

# Fish and Invertebrates at a Wetland Restoration Site in the Duwamish River Estuary, Seattle, Washington

Results of Biological Monitoring at Turning Basin Number  
Three, 1999-2007

Jeffery R. Cordell  
Jason Toft  
Elizabeth Armbrust

Wetland Ecosystem Team  
University of Washington  
School of Aquatic and Fishery Sciences

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## Executive Summary

This report summarizes biological monitoring conducted between 1999 and 2007 at a 1.3 acre aquatic habitat restoration site that was created in 1999 at Turning Basin Number Three, River Mile 5.3, in the Duwamish Waterway, Seattle, Washington (designated the “Port of Seattle” site). In addition, an older adjacent 0.4 acre restoration site (“Coastal America” site) was monitored. Both sites consisted of a higher elevation restored cove (minimum elevation of +3.9’ MLLW at Port of Seattle and +6.8’ at Coastal America) and a lower elevation mudflat (down to 0’ MLLW). The goals of this study were to: (1) assess juvenile salmon use and diet at the restored sites and a reference site on the adjacent waterway; (2) determine abundance of benthic invertebrates important to juvenile salmon at the restored sites and at a reference site on the waterway channel; and (3) assess abundance of insects at the restored sites. Invertebrates were monitored at the sites in 1999 and yearly from 2004-2007 (Cordell et al. 2001, Cooksey 2006, Cordell et al. 2006). Fish were sampled 2004-2007 during the spring juvenile salmon outmigration period using enclosure nets (restoration sites) and a beach seine (adjacent channel reference site). All fish species were enumerated, and diets were sampled from juvenile salmon using non-lethal gastric lavage. Concurrent with fish sampling, invertebrates were sampled in restored and reference areas using core samplers (macrofauna, meiofauna) and in restored emergent and riparian vegetation using insect fallout traps. Average densities and taxa richness values for fish and invertebrates were compared using standard analysis of variance techniques (ANOVA). Comparisons of assemblage structure among sites and sampling years were conducted using nonmetric multidimensional scaling (NMDS) ordination, analysis of similarity (ANOSIM), and similarity percentage (SIMPER) analyses.

### Juvenile Salmon

Juvenile salmonids, comprised mostly of chum salmon, usually dominated the fish composition at all sites, but starry flounders, staghorn sculpins, shiner perch, and threespine sticklebacks were also abundant. Fish assemblages were significantly different among the sites, mainly due to few Chinook salmon, coho salmon, shiner perch, starry flounder, and steelhead trout at the Coastal America site. The sites also differed in fish taxa richness, with significantly more species occurring at the Port of Seattle site and fewer species at the smaller and higher elevation Coastal America site.

Chinook salmon densities at the reference beach seine and Port of Seattle site were not significantly different, but both of these sites had significantly higher densities than the Coastal America site. Despite very high chum salmon abundance at the Port of Seattle site in 2006, there were no significant differences in chum salmon densities among the three sites.

Instantaneous ration, a measure of prey amount, was significantly higher in Chinook salmon captured at the Port of Seattle site than at the reference beach seine site. For chum salmon, instantaneous ration was usually higher at the two restoration sites as compared to the beach seine site, but this difference was only significant in 2007.

In juvenile Chinook salmon diets, adult chironomid flies and other adult and immature insects were prominent. *Americorophium* amphipods and other crustaceans also occurred consistently, especially at the beach seine site. Polychaete worms, consisting primarily of the family Nereidae, were particularly prominent at the Port of Seattle restoration site.

Diets of juvenile chum salmon were similar to those of juvenile Chinook salmon in having consistent contribution of adult chironomids, but differed from Chinook salmon diets in having larger proportions of larval chironomids and other immature insects and fewer adult insects, and also in having harpacticoid and calanoid copepods in the diets.

Based on percent composition, NMDS analysis indicated that Chinook salmon diets from the Port of Seattle site were significantly different than those from the beach seine site, mostly due to high contributions by polychaete worms to the diets at the Port of Seattle site and high contributions by *Americorophium* spp. amphipods at the beach seine site. No significant site-related differences were found for chum salmon diets.

### Benthic Macroinvertebrates

Benthic macroinvertebrate taxa richness was not significantly different between the two lower elevation sites, but was higher at these sites than at the higher elevation Coastal America and Port of Seattle restored sites. At the latter two sites, there was a significant year effect, with higher taxa richness in later years as compared to earlier sampling years.

NMDS ordination analysis based on percent composition of benthic macrofauna showed that the higher elevation Port of Seattle and Coastal America sites had different invertebrate assemblages than those at the lower elevation mud flat sites. The higher elevation Coastal America and Port of Seattle sites had higher proportions of the polychaete worms *Hobsonia florida* and *Manayunkia aesturina*, while the lower elevation sites were characterized by the presence of *Americorophium* spp. amphipods, which were rare at the upper elevation sites. Also, samples from the first post-restoration sampling of the Port of Seattle site in 1999 grouped separately from the other samples, due to chironomid fly larvae and the cumacean crustacean *Nippoleucon hinumensis* in the 1999 samples.

Separate analyses were conducted on two taxa—*Americorophium* amphipods and nereid polychaetes—that were common in both benthic samples and salmon diets. Results indicated that *Americorophium* spp. were significantly more abundant at the lower elevation sites, while nereids were significantly more abundant at the higher elevation sites (except in 1999).

### Benthic Meiofauna

Although analyses found significant differences in taxa richness among sampling years at both lower and higher elevation sites, there were no patterns of increasing or decreasing taxa richness across years.

Similar to the results from the benthic macrofauna samples, on the ordination plot the higher elevation Coastal America and Port of Seattle samples clustered separately from the lower elevation mudflat samples, and samples from the 1999 Port of Seattle site grouped separately. These differences were due to higher contributions of harpacticoids at the lower elevation sites, and by harpacticoid copepod nauplii (larval) stages and rotifers at the 1999 Port of Seattle site.

Separate analyses were conducted on harpacticoid copepods, which were common to both juvenile chum salmon diets and meiofauna samples. They were much more abundant at the lower elevation sites with the exception of 1999, when there was a large recruitment of harpacticoids at the higher elevation Port of Seattle site. At all of the sites

except for the higher elevation Coastal America site, the 1999 sample year had significantly higher harpacticoid densities than any other year.

### Fallout Insects

At both Coastal America and Port of Seattle sites, taxa richness of fallout insects significantly increased between the first sampling in 1999 and the samples taken in 2004-2005. Sampling stratum effect was also significant, with riparian vegetation strata having greater taxa richness values than emergent vegetation strata.

Although overall NMDS results were not significant, emergent vegetation strata sampled in 1999 clustered separately from other samples, and pairwise comparisons by year indicated that samples from 1999 were different than those from other sampling years. The difference between 1999 fallout trap samples and those from other sampling years was due to higher contributions by dipteran flies in 1999, and higher contributions by Acari (mites) and collembolans in other sampling years.

Aphids and adult chironomid flies were common to both fallout trap samples and juvenile salmon diets, and were analyzed separately. There were no evident trends of increasing or decreasing abundances of aphids across sampling years, and there was considerable among site- and year variation. Likewise, there were no evident trends of increasing or decreasing abundances of chironomids across sampling years.

### Conclusions

Results from this study indicate that the Coastal America and Port of Seattle Turning Basin restoration sites are providing significant habitat attributes for juvenile salmon. The Port of Seattle site had similar or higher salmon densities and fish taxa richness compared to the non-restored channel site. The Coastal America site, while having lower taxa richness and low densities of Chinook salmon, had densities of chum salmon comparable to the other sites. The salmon accessing the restoration sites were obtaining prey typical of that found in other restored and natural habitats in the region. In addition, high measures of instantaneous ration in fish using the restored sites suggests that the salmon there acquire prey in amounts exceeding those in the main river channel.

At the Port of Seattle site, benthic invertebrate taxa richness increased significantly between the first sampling event in 1999 and the next one in 2004, to levels comparable to those at the older Coastal America site. At both sites, total invertebrate densities in this study fell within the range of densities of benthic invertebrates previously found at other restored and natural reference sites in the region, but differed from those sites in having fewer chironomids and other insect larvae. The reason for this may be because organic matter has not yet built up at the sites.

Initial colonization of meiofaunal harpacticoid copepods at the Port of Seattle site was rapid: meiofaunal taxa richness reached stable levels within the first sampling year, and density data showed a large recruitment of harpacticoids at the Port of Seattle site just after construction in 1999.

Insects also apparently colonized both of the restoration sites quickly, with significant increases in taxa richness between the first sampling in 1999 and the next sampling in 2004. Planted riparian areas at the restoration sites had especially high taxa richness, which may have resulted from the diverse riparian plant assemblage that has developed.

Overall results indicate that the restoration sites at the Turning Basin area of the Duwamish Waterway are productive and contribute new sources of prey for juvenile salmon feeding in the transition zone of the Duwamish estuary. Several physical factors that are important in how the restoration sites have developed, and that should be addressed in the design of future restoration sites, include: (1) tidal elevation of created features, (2) size of created areas, and (3) access of restored sites to the main waterway. These physical factors appear to have strong influences on restored sites, regardless of restoration site age. Given the absence of natural habitat in this industrialized estuary, the best strategy for restoration may be to provide a diversity of sites that will provide natural features that are different from the existing heavily altered and channelized shoreline. Continued monitoring of the development and long-term stability of restored sites will help inform the design of improved sites for future restoration.

## Table of Contents

List of Figures .....	7
List of Tables .....	8
Introduction.....	9
Methods .....	12
Sampling Periods.....	12
Juvenile Salmon Densities and Diet.....	12
Fallout Insects .....	18
Benthic Macro- and Meiofauna .....	19
Statistical Analysis .....	19
Results .....	20
Environmental Parameters.....	20
Fish Composition and Juvenile Salmon Densities .....	20
Salmon Diets .....	25
Benthic Macroinvertebrates .....	31
Benthic Meiofauna .....	36
Fallout Insects .....	40
Discussion.....	44
Fish Assemblages .....	44
Juvenile Salmon Diets .....	45
Benthic Macroinvertebrates .....	45
Benthic Meiofauna .....	47
Fallout Insects .....	47
Conclusions.....	48
Acknowledgements .....	49
Literature Cited .....	50

## List of Figures

Figure 1. Schematic of Turning Basin restoration site showing habitat strata and sampling sites.....	12
Figure 2. Deploying the enclosure net at Turning Basin Port of Seattle Restoration site.....	14
Figure 3. Pole-seining within the enclosure net at Turning Basin Number Three.....	15
Figure 4. Fyke net at Coastal America restoration site.....	15
Figure 5. Beach seining at the reference Site. ....	16
Figure 6. Captured juvenile salmonids. ....	16
Figure 7. Measuring forklength of a juvenile salmonid. ....	17
Figure 8. Sampling juvenile salmon diet with gastric lavage.....	17
Figure 9. Insect traps deployed at Turning Basin Number Three. ....	18
Figure 10. Average taxa richness of fish assemblages at Turning Basin restoration and reference sites, 2004-2007.....	22
Figure 11. Density of Chinook salmon at Turning Basin restoration and reference sites, 2004-2007, $\pm$ SE. .....	23
Figure 12. Density of chum salmon at Turning Basin restoration and reference sites, 2004-2007, $\pm$ SE.....	23
Figure 13. NMDS ordination plot of fish percent composition from Turning Basin restoration and reference sites, 2004-2007. ....	25
Figure 14. Results from SIMPER analysis of taxa contributing to differences in fish assemblages among Turning Basin restoration and reference sites, 2004-2007. ....	25
Figure 15. Instantaneous ration for juvenile Chinook salmon captured at Turning Basin restoration and reference sites, 2004-2007. At the Coastal America site, Chinook salmon were captured only in 2006.....	26
Figure 16. Instantaneous ration for juvenile chum salmon captured at Turning Basin restoration and reference sites, 2004-2007. There was no data in 2005 due to low fish numbers. ....	27
Figure 17. Composition of juvenile Chinook salmon diets at Turning Basin restoration and reference sites, 2004-2007.....	28
Figure 18. Composition of juvenile chum salmon diets at Turning Basin restoration and reference sites, 2004-2007. There was no data in 2005 due to low fish numbers. ....	28
Figure 19. NMDS ordination plot of Chinook salmon diet based on percent gravimetric composition from Turning Basin Port of Seattle and beach seine reference sites, 2004-2007.....	29
Figure 20. Results from 2-way SIMPER analysis showing relative proportions of major taxa in Chinook salmon diets at Turning Basin restoration and beach seine reference sites, 2004-2007.....	30
Figure 21. NMDS ordination plot of chum salmon diet based on percent gravimetric composition from Turning Basin restoration and reference sites, 2004-2007.....	31
Figure 22. Average taxa richness by year of benthic macrofauna at Turning Basin restored and reference sites, 1999 and 2004-2007.....	32
Figure 23. Average densities of major benthic macrofauna taxa at Turning Basin restored and reference sites, 1999-2007. ....	33
Figure 24. NMDS ordination plot of macrofaunal invertebrate assemblages based on percent composition from Turning Basin restoration and reference sites, 1999 and 2004-2007.....	33
Figure 25. Results from 2-way SIMPER analysis showing relative proportions of major benthic macrofauna taxa at Turning Basin lower elevation restoration and reference sites, 1999-2007. ....	34
Figure 26. Density of <i>Americorophium</i> amphipods at Turning Basin restoration and reference sites, 1999 and 2004-2007, $\pm$ SE.....	35
Figure 27. Density of nereid polychaetes at Turning Basin restoration and reference sites, 1999 and 2004- 2007, $\pm$ SE. ....	36
Figure 28. Average taxa richness by year of benthic meiofauna at Turning Basin restored and reference sites, 1999 and 2004-2007.....	37
Figure 29. NMDS ordination plot of meiofaunal invertebrate assemblages based on percent composition from Turning Basin restoration and reference sites, 1999 and 2004-2007.....	38
Figure 30. Results from 2-way SIMPER analysis showing relative proportions of major benthic meiofauna taxa at Turning Basin higher elevation restoration and reference sites, 1999-2007.....	38
Figure 31. Density of harpacticoid copepods (naupliar stages omitted) at Turning Basin restoration and reference sites, 1999 and 2004-2007, $\pm$ SE. ....	39

Figure 32. Percent composition by numbers of harpacticoid copepod taxa at Turning Basin restoration and reference sites, 1999 and 2004-2007. ....	40
Figure 33. Average taxa richness by year of fallout trap invertebrates at Turning Basin restored sites, 1999 and 2004-2007.....	41
Figure 34. NMDS ordination plot of fallout trap invertebrate assemblages based on percent composition from Turning Basin restoration sites, 1999 and 2004-2007.....	42
Figure 35. Results from SIMPER analysis showing relative proportions of major emergent vegetation fallout trap invertebrate taxa at Turning Basin higher elevation restoration sites, 1999-2007.....	42
Figure 36. Density of Aphididae at Turning Basin restoration vegetation strata, 1999 and 2004-2007, $\pm$ SE. ....	43
Figure 37. Density of Chironomidae at Turning Basin restoration vegetation strata, 1999 and 2004-2007, $\pm$ SE. ....	44

## List of Tables

Table 1. Averages of salinity, temperature, and other environmental measurements at Turning Basin restored and reference sites, 2004-2007. ....	21
Table 2. Percent composition of fish species captured at Turning Basin restored and reference sites, 2004-2007.....	21
Table 3. Average lengths and wet weights of juvenile Chinook and chum salmon caught at Turning Basin restoration and reference sites, 2004-2007.....	24



## Introduction

The Duwamish River estuary is the highly industrialized lowest reach of the Duwamish River located in Seattle, Washington. Over the last 150 years, Seattle has become a densely populated urban center, resulting in loss of 98% of Duwamish River estuarine delta wetlands, which have been replaced with over 2,100 ha of developed shorelines and floodplain (Simenstad et al. 2005). The Duwamish watershed has also been reduced significantly: at one time, the estuary received the combined flows of three major watersheds covering almost 4,250 km<sup>2</sup> (Collins and Sheikh 2005). Two of these tributaries have been permanently diverted elsewhere, resulting in loss of 70-75% of the historic freshwater inflow to the estuary. Over the last century, pollution in the Duwamish River estuary changed from urban and resource-based contaminants to industrial chemicals, and despite pollution control regulations and contaminant remediation, earlier contamination remains, and the estuary is a major Superfund (Comprehensive Environmental Response, Compensation, and Liability Act [CERCLA]) site.

Despite problems with habitat quantity and quality, restoration of natural functions in the Duwamish River estuary is a high priority for trustees of damaged public resources (Simenstad et al. 2005). Wetland restoration in the estuary began in 1988, as compensatory mitigation for development and historic damage, which resulted in ~6 ha of rehabilitated habitat. These and later mitigation/restoration projects mainly focused on habitats that provide juvenile salmon with food, refuge from predation, and brackish waters for physiological adaptation. Location of most sites were based on opportunistic criteria (e.g., property availability and cost) rather than ecological context (Simenstad et al. 2005), and created habitats consisted mainly of removal of shoreline armoring and other structures in middle and upper intertidal elevations, excavating off-channel features, and planting emergent and riparian vegetation. Between 1992 and 1996, the Federal Coastal America Program funded the first non-regulatory restoration actions in the estuary, coordinated by several federal and local agencies. These sites only comprised 0.5 ha, but they provided the foundation for two clusters of restoration sites that have emerged in the estuary (Simenstad et al. 2005). After 2000, Elliott Bay/Duwamish River CERCLA actions began appearing, expanding the dimensions and distribution of restoration sites in the estuary. While biological monitoring of some of these sites has been conducted, the technical expertise and expense required to do so have precluded regular post-construction monitoring in many cases. One exception has been at a suite of sites in the Duwamish Waterway that have been monitored for sediment structure, invertebrates, fish, and avifauna, beginning before the sites were constructed in 1993 (summarized in Cordell et al. 2001). Three of these were the original Coastal America Program sites, and biological monitoring of them was provided for by a consortium of agencies, including the Port of Seattle, U.S. Fish and Wildlife Service (USFWS), U.S. Army Corps of Engineers (USACE), and U.S. Environmental Protection Agency (USEPA). Monitoring of the original three Coastal America sites was completed in 1999, but additional sites were added in the late 1990s and early 2000s by the Port of Seattle and the Elliott Bay/Duwamish Restoration Panel (see <http://www.darrp.noaa.gov/northwest/elliott/restore.html> for site summaries of the Restoration Panel sites), which included support for biological monitoring of the sites.

This report details results of biological monitoring over an eight-year period at one of these sites.

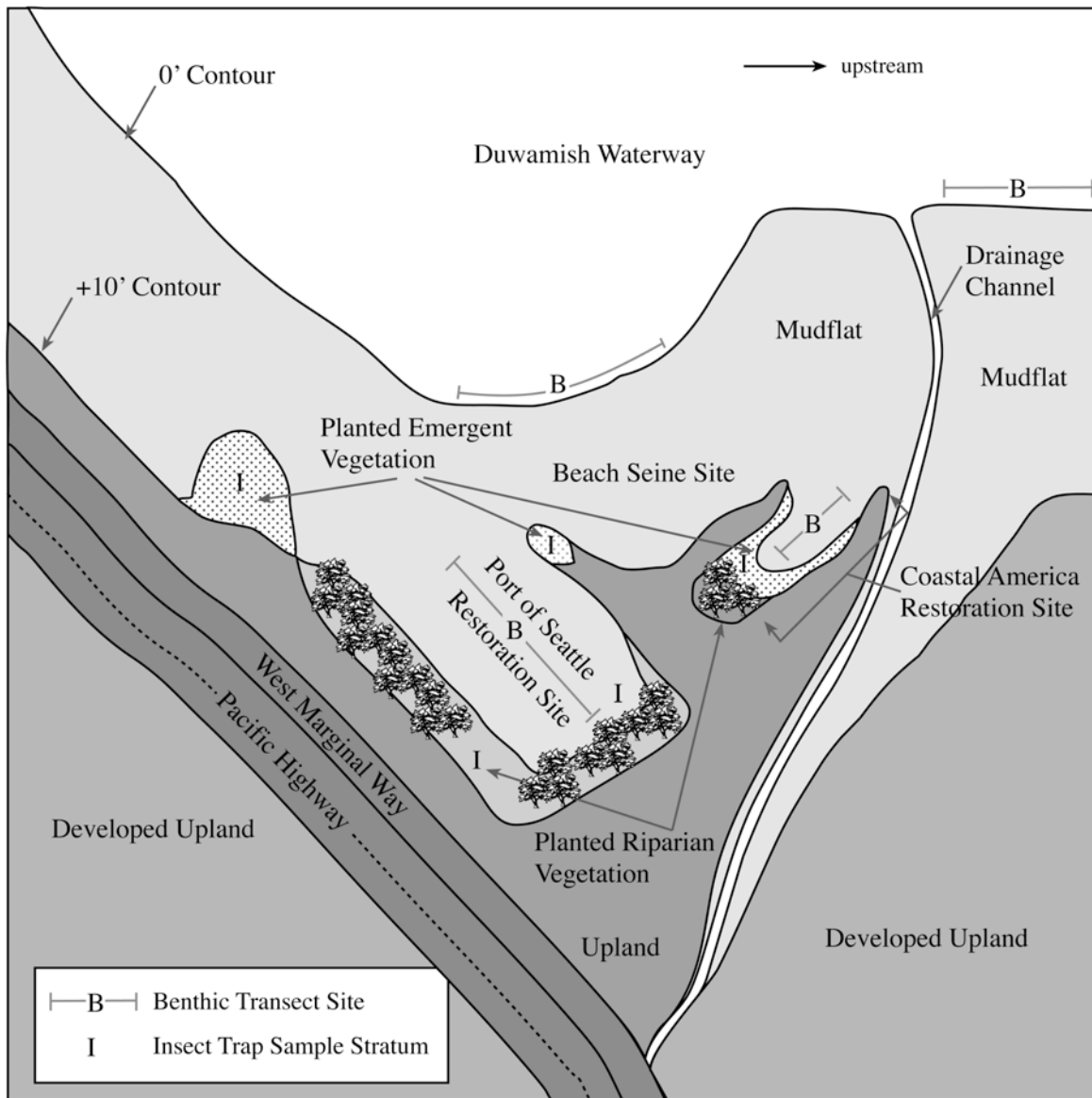
One of the primary goals of all of the Duwamish River estuary restored habitat monitoring studies was to measure habitat function for juvenile salmon at the restored sites. The majority of this monitoring has involved indirect measures of productivity, such as amounts of potential prey invertebrates at the sites. This type of “opportunity” measure appraises the capability of juvenile salmon to access and benefit from a habitat, but does not determine if salmon will use the site or derive any realized function (e.g., growth and survival) from it (Simenstad and Cordell 2000). In some cases, actual use of the sites by juvenile salmon was documented, but the studies mainly concentrated on measuring capacity metrics for juvenile salmon (see Simenstad and Cordell 2000, Cordell et al. 2001, Cordell et al. 2003, Simenstad et al. 2005, Cordell et al. 2006, Cordell et al. 2007). Several methodologies that measured densities of invertebrate juvenile salmon prey taxa proved useful, and in this study we adopted those techniques, along with sampling of juvenile salmon and their stomach contents to make post-construction assessment of the Port of Seattle’s Turning Basin Number Three restoration site in the Duwamish Waterway.

In 1999 the Port of Seattle completed approximately 1.3 acres of aquatic habitat restoration at Turning Basin Number Three, located at river mile 5.3 in the Duwamish Waterway as a compensatory action resulting from container cargo pier construction at Terminal 5 in southwest Elliott Bay (see web link above for a map showing location of the Duwamish Waterway Turning Basin). This site occurs in one of the above-mentioned restored habitat clusters in the estuary, and is connected to an earlier Coastal America Program habitat restoration site (Fig. 1). The restoration area includes the following features: (1) approximately 1.3 acres of intertidal mud and sand substrate, with a cove that dewateres at +3.9’ MLLW; (2) approximately 0.44 acres of emergent vegetation; and, (3) approximately 0.64 acres of riparian vegetation (Fig. 1). Recent evaluations indicate that the restoration area substrate and vegetation areas are stable as constructed.

The Port of Seattle required outside professional services for the purpose of evaluating aquatic resource attributes of importance to juvenile salmon at the Turning Basin Number Three restoration site. In particular, the Port was interested in: (1) assessing juvenile salmon use and diet; (2) determining abundance of benthic invertebrate food items important to juvenile salmon; (3) assessing abundance of drift insect food items; and (4) analyzing and evaluating data from multiple years of sampling. Similar single-year assessments have been conducted at the site in 1999, 2004, 2005, and 2006 (Cordell et al. 2001, Cooksey 2006, Cordell et al. 2006, Cordell et al. 2007). In this report, we present and summarize an analysis of the complete data set from biological monitoring of the Turning Basin Number Three site from 1999-2007.

The goals of the monitoring efforts summarized in this report were to quantify insects and benthic invertebrates at the Coastal America and Port of Seattle Turning Basin restored sites, and to capture juvenile salmon and sample their stomach contents at the sites. The Coastal America site was included for comparison purposes, because it is an older site (created in 1995) and other natural reference emergent habitats are virtually nonexistent in the Duwamish River estuary. The Coastal America site is smaller and at a higher elevation, being 0.4 acres in size and having a intertidal cove that dewateres at +6.8’ MLLW. Insects produced from riparian and emergent vegetation are often

important prey of juvenile salmon foraging in Pacific Northwest estuaries (summarized by Simenstad et al. 1991; Shreffler et al. 1992, Miller and Simenstad 1997, Brennan et al. 2004, Toft et al. 2007), including the Duwamish Waterway (Cordell et al. 2001, 2006, 2007). Fallout insects would probably comprise a greater proportion of the diet of juvenile salmon in the Duwamish Waterway if there were more riparian and emergent vegetation present, and most of the restoration sites in the waterway (including the Turning Basin Number Three site) incorporate new vegetation plantings toward this end. Benthic invertebrates that are important prey taxa for juvenile salmon in Pacific Northwest estuaries include *Americorophium* and *Eogammarus* spp., larvae of dipteran flies, and harpacticoid copepods (Simenstad et al. 1991, Levings and Nishimura 1997, Cordell et al. 1999, 2001, 2006, 2007). Therefore, many of the restoration sites in the Duwamish waterway, including the Turning Basin sites, also include areas of emergent mud- or sand flats as part of their design.



**Figure 1. Schematic of Turning Basin restoration site showing habitat strata and sampling sites.**

## Methods

### Sampling Periods

The first sampling occurred in Spring 1999, several months after construction of the site. In this year, only benthic and fallout invertebrates were sampled. Funding for biological monitoring was unavailable from 2000-2003, but from 2004-2007 both fish and invertebrates were sampled at the site each year.

### Juvenile Salmon Densities and Diet

From 2004-2007, juvenile salmon densities and diets were sampled at three locations in the Turning Basin area (Fig. 1). Juvenile salmon were captured and enumerated at (1) the large Port of Seattle restoration site (the 1999 mitigation site, which shall henceforth be referred to as the Port of Seattle site); (2) the adjacent, smaller Coastal America restoration site (completed in 1995); and, (3) a mud and sand substrate intertidal location

adjacent to both restoration sites (reference location). Sampling occurred on four dates between April and June, a period that encompasses the juvenile salmon outmigration. The restoration sites de-water at mid-tide, and are only accessible by fish at relatively high tides. Fish sampling took place during these inundation periods at each restoration and reference site, using enclosure nets (Toft et al. 2007).

Nets were deployed during high spring tides, and were sampled for fish as the sites de-watered at low tides. The net used at the Port of Seattle site was 60-m long and 4-m deep, with a 0.64-cm mesh net (Figs. 2, 3). A smaller fyke-type net was deployed similarly at the Coastal America site (Fig. 4). At the restoration sites the net was used to block the mouth of the restoration site, completely enclosing each site. Fish were removed with either a small pole seine (9.1-m. x 1.2-m., 0.64-cm mesh) or dip nets as the tide receded, usually starting at mid-tide several hours after net deployment. All fish were removed before low tide. Numbers were converted to densities by standardizing catches to the surface area sampled with the nets. For the restoration sites area was calculated by digitizing the specific sampling areas blocked with enclosure nets from digital orthophotos and GPS points. The resulting areas used were as follows: Port of Seattle 2000 m<sup>2</sup>; Coastal America 500 m<sup>2</sup>. At the reference site, fish were sampled with a beach seine (37-m. x 2-m., 30-m lines, 0.2-cm mesh bag) set from a small boat (Fig. 5). The area sampled by the beach seine was calculated to be 520 m<sup>2</sup>.

After enclosure net deployment, the following environmental measurements were taken: (1) surface and bottom water salinities and temperatures were recorded with a portable YSI meter, (2) total amount of time the net was deployed before completed fish sampling, and (3) maximum water depth at time of net deployment at high tide.

Non-salmonid fish captured in the net were identified, counted, and released. Captured juvenile salmonids were identified and enumerated, and a subsample measured for fork length and weight (Figs. 6, 7). Diets of juvenile Chinook salmon from enclosure nets were sampled by gastric lavage (Fig. 8). This method consisted of placing fish in a tray of seawater with a small amount of the anesthetic MS-222 for approximately 30 seconds. Each fish was then measured for fork-length and weight. Gut contents were removed using a modified garden pump sprayer with a custom nozzle and filtered seawater. Gastric lavage has been shown to result in 100% removal of food items and to have no adverse long-term effects in salmonids (Twomey and Giller 1990). Contents were washed into a 106- $\mu$ m sieve and fixed in 10% buffered formaldehyde solution. Fish were immediately placed in a bucket of ambient water for recovery (approximately 2-3 minutes), and then released. Diets of juvenile chum salmon from the enclosure nets were obtained from whole fish samples, which were euthanized in MS-222 and then fixed in 10% formalin. A total of 219 Chinook and 274 chum salmon diets were analyzed.

In the laboratory, salmonid prey items were identified using a dissecting microscope. Small benthic and planktonic crustaceans and a few other taxa were identified to genus or species. For other major prey items such as insects, identification was only practical to the order or family level. Each prey taxon was counted and weighed to the nearest 0.0001g.

Instantaneous diet ration was calculated for each fish analyzed, as grams of prey wet weight divided by fish wet weight. In some cases juvenile Chinook salmon lengths were taken but weights were not recorded (13% of the Chinook sampled for diets): in these

cases a weight was calculated based on a length-weight regression generated from all Chinook salmon subyearlings caught at the Turning Basin 2004-2007.



**Figure 2. Deploying the enclosure net at Turning Basin Port of Seattle Restoration site.**





**Figure 3. Pole-seining within the enclosure net at Turning Basin Number Three.**



**Figure 4. Fyke net at Coastal America restoration site.**





**Figure 5. Beach seining at the reference Site.**



**Figure 6. Captured juvenile salmonids.**





**Figure 7. Measuring forklength of a juvenile salmonid.**



**Figure 8. Sampling juvenile salmon diet with gastric lavage.**

### **Fallout Insects**

The three locations described above were evaluated for potential drift insects using insect fallout traps. Fallout invertebrates were sampled in 1999 and each year from 2004-2007. Traps were located in riparian and emergent plant areas at each site, sampled during four dates between April and June overlapping with the fish sampling (Fig. 1). At the Port of Seattle site, traps were placed in three emergent vegetation areas: (1) the land-side point at the mouth of the site (designated “Port Little Point Emergent”, (2) the river-side point at the mouth of the site (Port Big Point Emergent), and at the head of the restoration site (Port Cove Emergent). Fallout traps, which consist of plastic storage bins with about 4 cm of soapy water in the bottom, have been used extensively in previous studies of Duwamish Waterway restoration sites (Fig. 9; Cordell et al. 2001, 2003, 2007). They are designed to catch insects that fall from the air or from riparian vegetation and as such measure direct input of insects to the aquatic system. Five replicate traps were placed haphazardly in or near vegetation at each sampling stratum for a period of three days. At the end of the three-day period, each tray was drained through a 106 $\mu$ m mesh sieve, and the insects were washed into a sample jar and fixed in 70% isopropanol solution. In the laboratory, insects were identified to family level for important salmonid prey taxa, and to order level for the remainder.



**Figure 9. Insect traps deployed at Turning Basin Number Three.**

### **Benthic Macro- and Meiofauna**

Benthic core samples were collected from mud and/or sand flats at the three sites, corresponding with the insect sampling dates. Samples were also taken at the 0.0 feet MLLW elevation restoration and reference sites sampled in previous Coastal America monitoring projects (Fig. 1). We used protocols for sampling macro- and meiobenthos in the Duwamish Waterway that have recently been used extensively in the Duwamish Waterway (Cordell et al. 2001, 2003, 2007). They consist of a 2-inch diameter ( $0.0024 \text{ m}^2$ ) pvc plastic core taken to a depth of 10 cm for macrofauna and a 1-inch diameter ( $0.0002 \text{ m}^2$ ) pvc plastic core taken to a depth of 10 cm for meiofauna. For each stratum, 10 replicate cores of each type were taken. Samples were fixed in the field in 10% buffered formaldehyde solution. In the laboratory, macrofauna samples were washed through a 0.5 mm mesh sieve, and meiofauna samples were washed through a 0.106 mm sieve. Important salmon prey invertebrates were identified to genus or species with the exception of insects, which were identified to family level; other taxa were identified to order level.

### **Statistical Analysis**

Data was entered in Microsoft Excel and analyzed using S-plus (univariate statistics) and Primer version 6 (multivariate statistics) software (Clarke and Warwick 2001). Analysis of variance (ANOVA;  $\alpha = 0.05$ ) was conducted on densities and taxa richness of fish and invertebrates and instantaneous ration of fish diets (weight of prey divided by weight of fish). Main tests were typically 2-way ANOVAs with interactions (e.g. Site x Year). Fish densities were first log-transformed to satisfy assumptions of normality and homogeneous variances. For significant results, Tukey's test for multiple comparisons was used to identify specific differences between means. All density graphs display standard error bars.

Percent compositions of fish, fish diets, and invertebrates were analyzed with nonmetric multidimensional scaling (NMDS) ordination, analysis of similarity (ANOSIM), and similarity percentage (SIMPER) analysis. These analyses uncover patterns in multivariate groupings of the data, and have been widely used when analyzing datasets with multiple species compositions including fish, fish diets, and invertebrates (Platell and Potter 2001, Schafer et al. 2002, Desmond et al. 2002, Levin and Talley 2002, Valesini et al. 2004, Wildsmith et al. 2005). Fish samples were averaged to overcome the problem of variable individual net catches. For diet data, percentages of gravimetric composition for each prey taxon per fish were used for analysis. We used methods similar to those of Schafer et al. (2002) and Platell and Potter (2001), in which individual diet data is randomly allocated into groups of five (four or three if not an exact break) and the means calculated for each group. Such averaging prior to multivariate analysis overcomes the problem of individual fish stomachs not being representative of the total diet. Taxa resolution in diets was set at a level consistent across years to alleviate any differences of variable digestion and corresponding levels of species identifications, and non-animals such as sediment, plant matter, and unidentified material were removed prior to analysis. All macrofauna, meiofauna, and insect samples were included separately in analyses, stated as percent composition of taxa within each sample.

For ordination, percentages were square-root transformed, and species accounting for less than 3% of the abundance of any one sample excluded. NMDS results were plotted

on two dimensional charts in multidimensional space based on a Bray-Curtis similarity matrix (the axes have no scale), to illustrate differences in taxa assemblages. Stress is a measure of how well the ordination plot displays the data, values under 0.2 were considered useful (Clarke and Warwick 2001). ANOSIM gives a p-value similar to an ANOVA, with values of  $p < 0.05$  indicating significance. ANOSIM also generates a value R scaled between -1 and +1, with zero representing no difference among a set of samples, and values approaching 1 and -1 indicating greater biological importance in the differences. Typically, low values of R around 0.1 to 0.2 indicate minimal biological importance even if p values show significance, especially in datasets with many replicates, whereas values approaching 0.4 and above indicate meaningful biological importance (Clarke and Warwick 2001). R values are therefore more useful in interpreting results than p values, as high R values are not a function of the number of replicates. Thus, when R values were around 0.1 to 0.2, results were considered not biologically significant, and further analysis was not conducted. When significant differences were indicated with ANOSIM, a SIMPER analysis was used to identify the taxa that accounted for the differences, using a cutoff value of 5% (i.e., only taxa representing >5% contribution to significant differences were used). SIMPER generates a ranking of the percent contribution by taxa that contribute to the significant differences between factors.

## **Results**

### **Environmental Parameters**

The Port of Seattle and Coastal America sites had similar average surface salinities, ~1 psu on the surface (Table 1). The bottom salinity was higher at the Port of Seattle site (annual average of 1.6 - 6.7 psu) as compared with the Coastal America site (1.6 - 1.8 psu). Average water temperatures were consistent among sites and sampling years, ~12-13 °C (Table 2). Average maximum water depth at the net was greater at the Port of Seattle site (annual average 1.3 – 1.8 m) than at the Coastal America site (0.7 – 1.0 m).

### **Fish Composition and Juvenile Salmon Densities**

Overall fish numerical composition from enclosure nets at the Port of Seattle site was dominated by juvenile chum salmon in 2006 and 2007, and by starry flounders in 2004 and staghorn sculpins in 2005 (Table 2). Other relatively abundant fish included shiner perch and sticklebacks. Juvenile Chinook salmon were usually a minor component of overall fish composition except in 2005, when they made up 18% of the catch. Fish proportions were similar at the reference beach seine site, with juvenile chum salmon dominating in 2006 and 2007. The main differences between the beach seine site and the Port of Seattle site were a large proportion of coho salmon caught at the beach seine site in 2004, and generally lower proportions of shiner perch caught at that site. At the smaller, higher elevation Coastal America restoration site, there were usually much higher juvenile chum salmon and much lower Chinook salmon proportions than at the other two sites. The Coastal America site also had very low proportions of shiner perch.

**Table 1. Averages of salinity, temperature, and other environmental measurements at Turning Basin restored and reference sites, 2004-2007.**

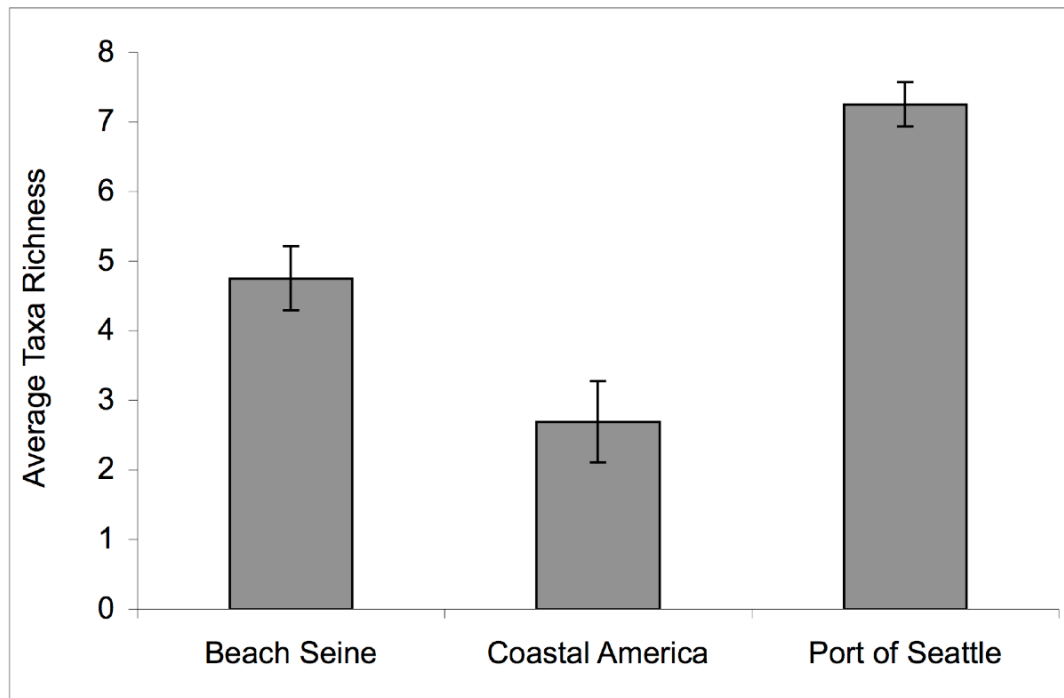
Site	Year	Surface Salinity (psu)	Bottom Salinity (psu)	Surface Temperature (°C)	Bottom Temperature(°C)	Time net deployed (hours)	Maximum Water Depth at Net (m)
Coastal America	2004					2.0	1.0
	2005	1.1	1.6	12.8	13.0	1.6	0.8
	2006	0.8	1.8	12.4	12.6	1.5	0.7
	2007	1.1	1.8	13.4	13.8	1.4	0.7
Port of Seattle	2004	1.1	5.8	12.6	13.0	3.5	1.8
	2005	0.9	1.6	12.9	12.9	2.8	1.4
	2006	0.7	3.8	12.4	12.2	3.4	1.3
	2007	1.1	6.7	12.3	12.6	2.9	1.5

**Table 2. Percent composition of fish species captured at Turning Basin restored and reference sites, 2004-2007.**

Site	Year	Chinook Salmon	Coho Salmon	Chum Salmon	Steelhead Trout	Staghorn Sculpin	Shiner Perch	Starry Flounder	Threespine Stickleback	Other Species
Beach Seine	2004	6.1%	53.5%	10.6%	2.0%	6.8%	6.3%	13.1%	0.6%	0.3%
	2005	12.6%	1.4%	12.3%	14.1%	16.8%	29.5%	12.5%	0.7%	0.1%
	2006	3.1%		35.2%	0.4%	20.0%	5.6%	33.4%	1.6%	0.7%
	2007	8.3%		53.1%		4.5%	1.6%	28.7%	3.6%	0.2%
Coastal America	2004			83.3%		14.0%		1.3%	0.7%	0.7%
	2005			60.0%		20.0%			20.0%	0.0%
	2006	0.7%		14.3%		25.6%	2.9%	3.6%	49.5%	3.4%
	2007	0.7%		86.7%		4.4%	1.5%	3.0%	3.7%	0.0%
Port of Seattle	2004	3.5%	0.2%	24.0%	0.1%	14.9%	16.0%	38.5%	2.4%	0.4%
	2005	18.0%	3.6%	5.9%	0.2%	43.7%	12.0%	11.3%	5.0%	0.3%
	2006	1.3%	0.1%	33.7%	0.0%	28.3%	11.3%	20.8%	4.5%	0.0%
	2007	0.9%	0.3%	26.5%	0.1%	14.8%	19.5%	22.3%	15.4%	0.3%



Average taxa richness of the fish assemblages was highest at the Port of Seattle restoration site, and lowest at the Coastal America site (Fig. 10). ANOVA indicated that the site differences were highly significant ( $p < 0.0001$ ) and that there was no year effect (i.e., results were consistent across years).



**Figure 10.** Average taxa richness of fish assemblages at Turning Basin restoration and reference sites, 2004-2007.

Standardized Chinook salmon densities were high at both the beach seine and Port of Seattle sites (Fig. 11). ANOVA indicated that site was a significant factor ( $p < 0.0001$ ), and year was not a significant factor ( $p = 0.31$ ). A Tukey post-hoc test for multiple comparisons indicated that both the Port of Seattle and beach seine sites had higher Chinook salmon densities than the Coastal America site, but were not significantly different from each other.

For chum salmon, standardized densities were fairly consistent across sites and years, except for high catches at Port of Seattle in 2006 (Fig. 12). ANOVA indicated that both site and year differences were not statistically significant ( $p = 0.094$  and  $0.11$ , respectively).

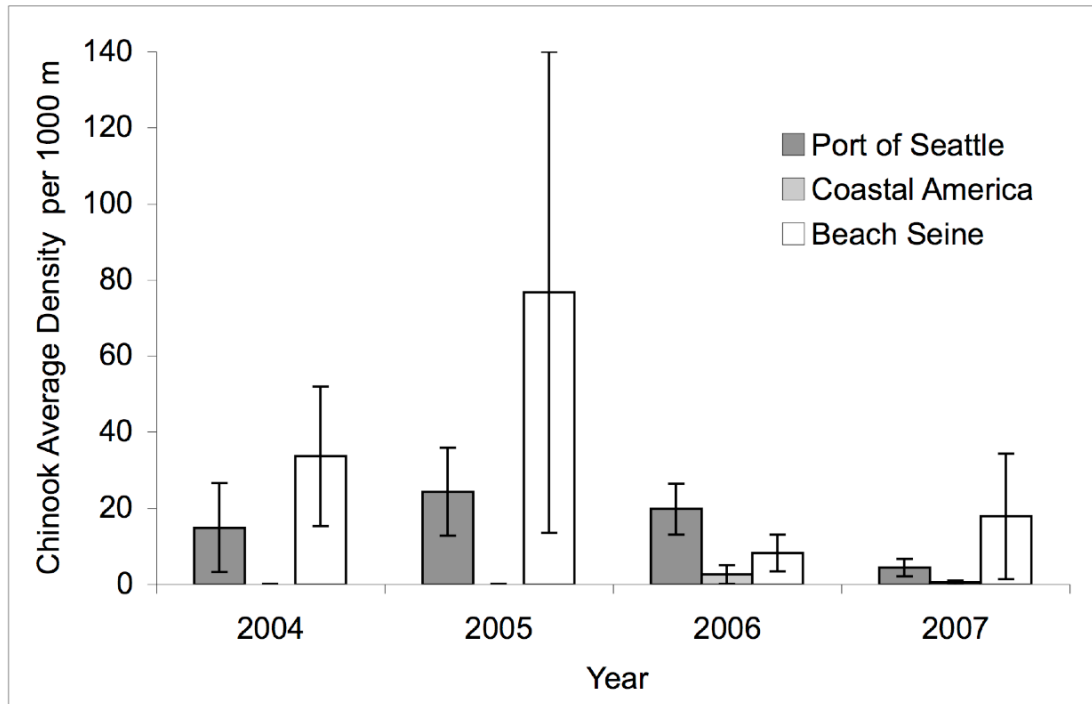


Figure 11. Density of Chinook salmon at Turning Basin restoration and reference sites, 2004-2007,  $\pm$  SE.

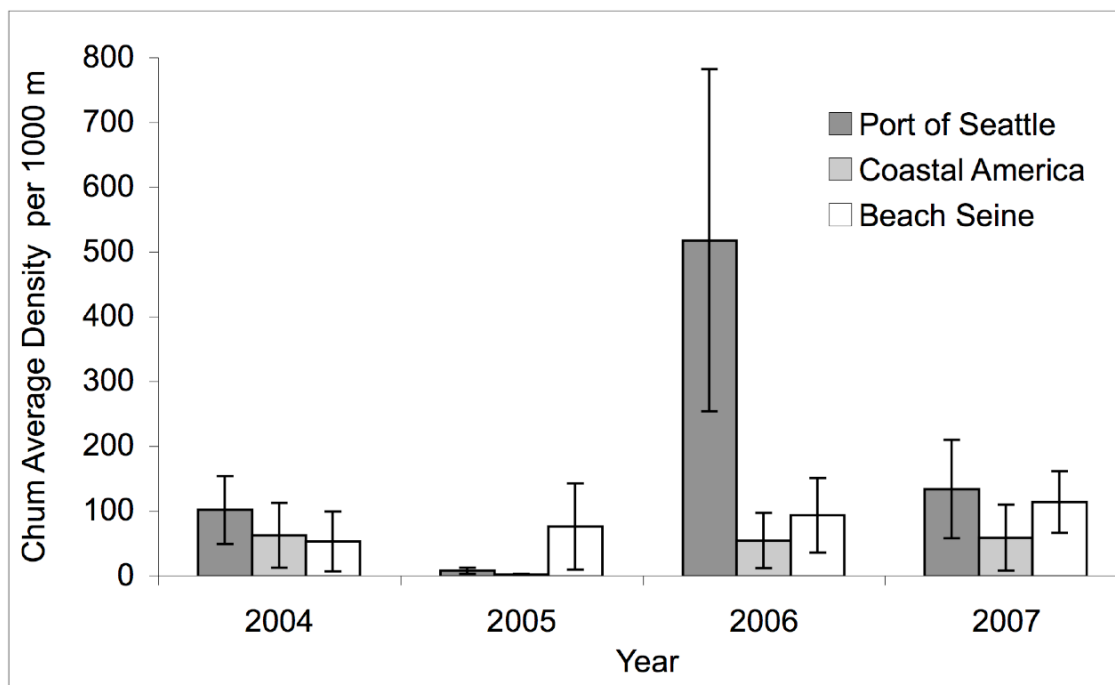


Figure 12. Density of chum salmon at Turning Basin restoration and reference sites, 2004-2007,  $\pm$  SE.

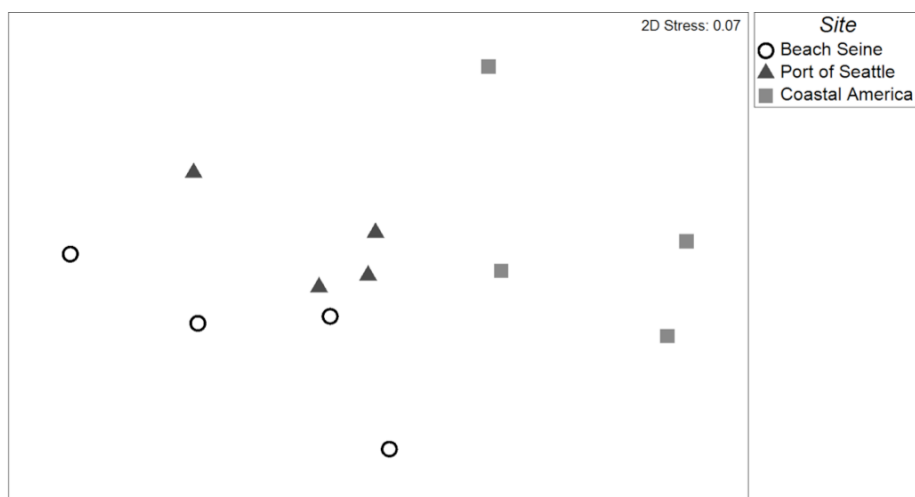
In three of the four sampling years, juvenile Chinook salmon from subsamples at the Port of Seattle restoration site were smaller than those taken from the reference beach seine (Table 3). Chum salmon subsampled at the Coastal America site were consistently smaller than those taken from the beach seine and Port of Seattle sites.

**Table 3. Average lengths and wet weights of juvenile Chinook and chum salmon subsampled at Turning Basin restoration and reference sites, 2004-2007.**

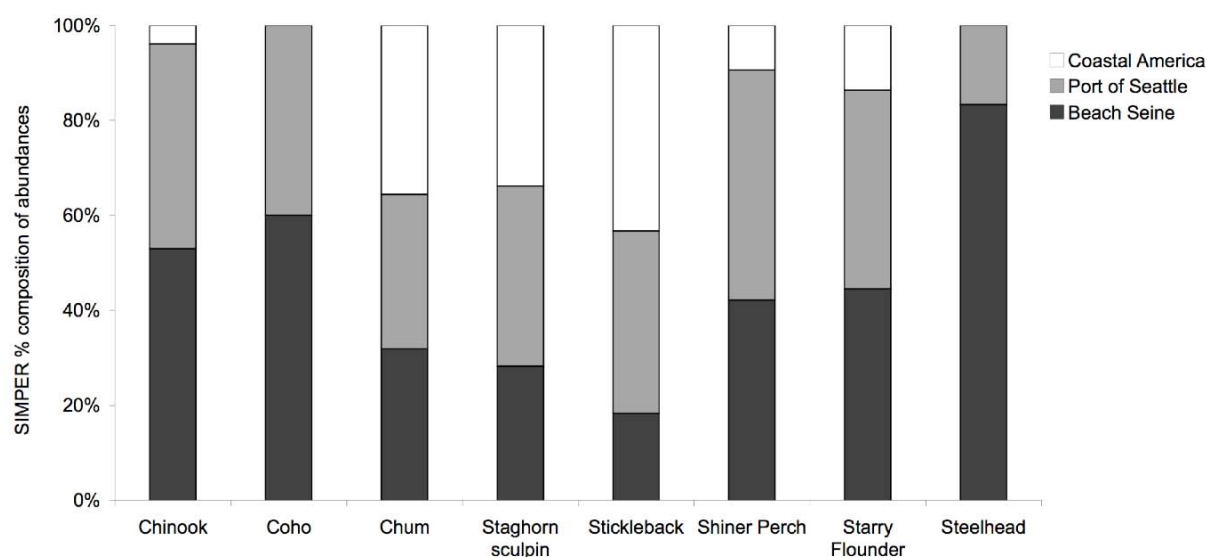
Species	Year	Site	Forklength (mm)	Weight (g)
Chinook	2004	Beach Seine	84.6	6.73
		Port of Seattle	82.2	6.40
	2005	Beach Seine	79.7	5.67
		Port of Seattle	80.3	5.89
	2006	Beach Seine	81.3	5.47
		Coastal America	75.2	4.56
		Port of Seattle	78.1	4.56
	2007	Beach Seine	81.4	5.70
		Port of Seattle	67.0	3.50
Chum	2004	Beach Seine	52.5	0.91
		Coastal America	45.8	0.65
		Port of Seattle	47.0	1.01
	2006	Beach Seine	46.1	1.02
		Coastal America	44.0	0.82
		Port of Seattle	51.6	1.34
	2007	Beach Seine	48.1	1.17
		Coastal America	40.0	0.58
		Port of Seattle	51.0	1.26

NMDS analysis of the fish assemblages based on percent composition proved to be a useful model according to statistical guidelines (stress less than 0.2 considered useful; Clarke and Warwick 2001), showing a NMDS ordination 2-d stress of 0.07 (Fig. 13). On the ordination plot, the Coastal America site fish samples clustered separately than those from the Port of Seattle and beach seine sites. These results were significant (global ANOSIM,  $R = 0.428$ ,  $p = 0.006$ ; pairwise comparisons of Coastal America between Port of Seattle and beach seine,  $R > 0.65$ ,  $p < 0.05$ ). A SIMPER analysis illustrated that the main pattern driving significant differences were low or no contributions of Chinook salmon, coho salmon, shiner perch, starry flounder, and steelhead trout at the Coastal America site (Fig. 14).





**Figure 13. NMDS ordination plot of fish percent composition from Turning Basin restoration and reference sites, 2004-2007.**



**Figure 14. Results from SIMPER analysis of taxa contributing to differences in fish assemblages among Turning Basin restoration and reference sites, 2004-2007.**

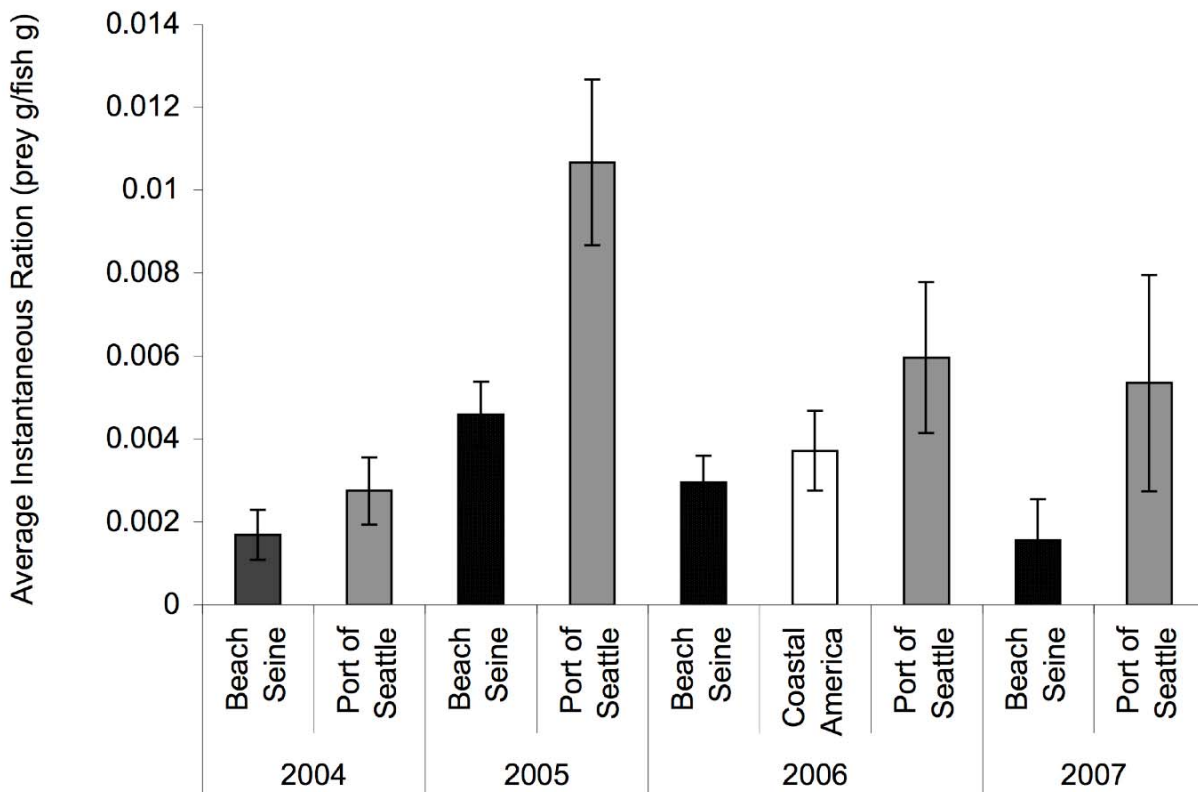
## Salmon Diets

### Instantaneous Ration

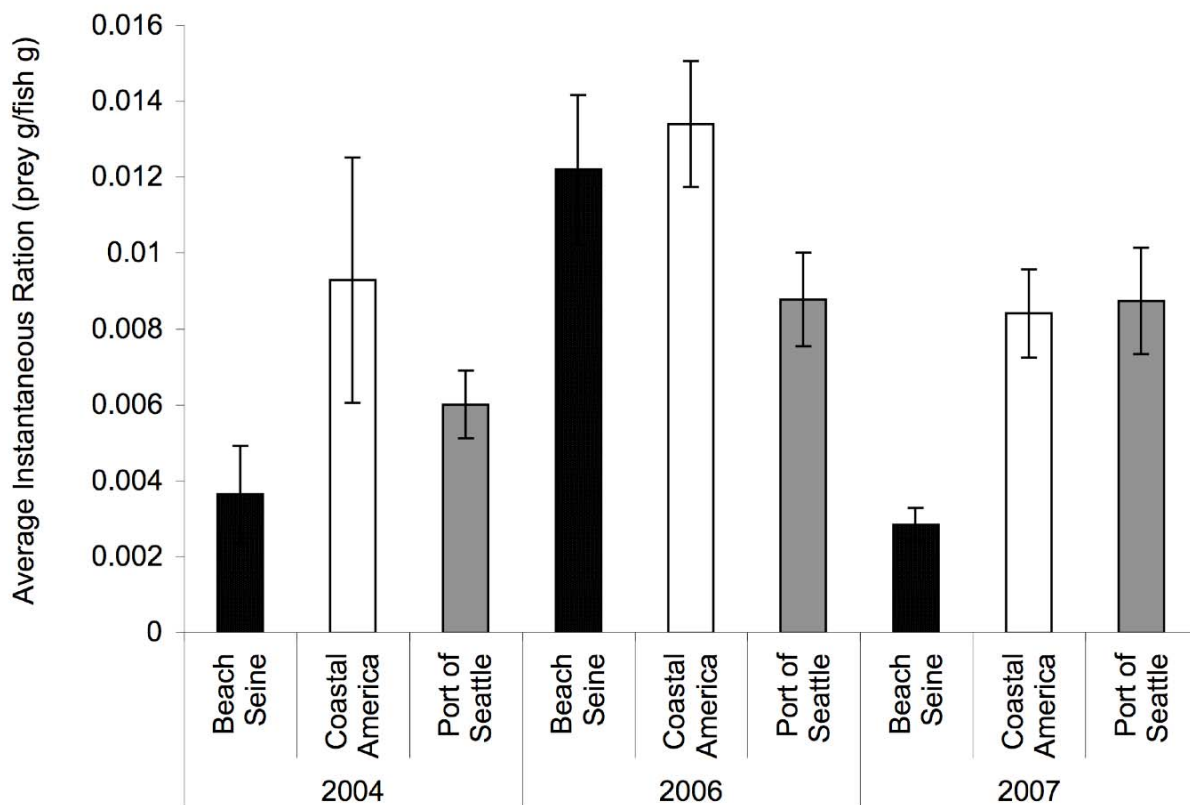
The average instantaneous ration of prey in juvenile Chinook salmon was highest in diets from the Port of Seattle site in each year (Fig. 15). In a 2-way site x year ANOVA, site effect was significant ( $p=0.005$ ) with the Port of Seattle site being significantly greater than the beach seine site, while year effect ( $p=0.6$ ) and interactions ( $p=0.9$ ) were not significant. In the one year (2006) in which juvenile Chinook salmon were caught at the Coastal America site, instantaneous ration was intermediate between values at the beach seine and Port of Seattle sites.

For juvenile chum salmon, average instantaneous ration was highest at the Coastal America site in two of the three years (2004, 2006) for which diet data was analyzed (Fig. 16). In 2007,

instantaneous ration was similar at the Port of Seattle and Coastal America sites, and higher than at the beach seine site. In a 2-way site x year ANOVA, overall site effect was significant ( $p=0.012$ ), while year effect was not significant ( $p=0.29$ ). There was a significant interaction effect ( $p=0.028$ ), and post-hoc ANOVA and Tukey tests indicated that differences among sites were not significant in 2004 and 2006 ( $p=0.16$ ,  $p=0.09$ , respectively). However, there was a significant site difference in 2007 ( $p=0.0007$ ), with both the Port of Seattle and Coastal America sites having significantly higher instantaneous rations as compared to the beach seine site.



**Figure 15. Instantaneous ration for juvenile Chinook salmon captured at Turning Basin restoration and reference sites, 2004-2007. At the Coastal America site, Chinook salmon were captured only in 2006.**

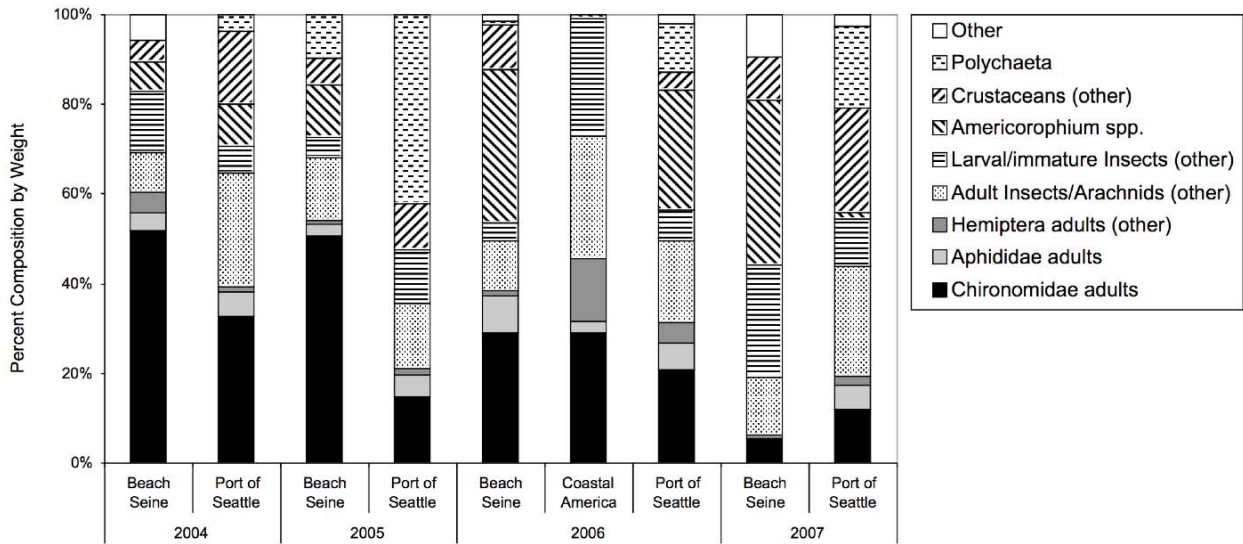


**Figure 16. Instantaneous ration for juvenile chum salmon captured at Turning Basin restoration and reference sites, 2004-2007. There was no data in 2005 due to low fish numbers.**

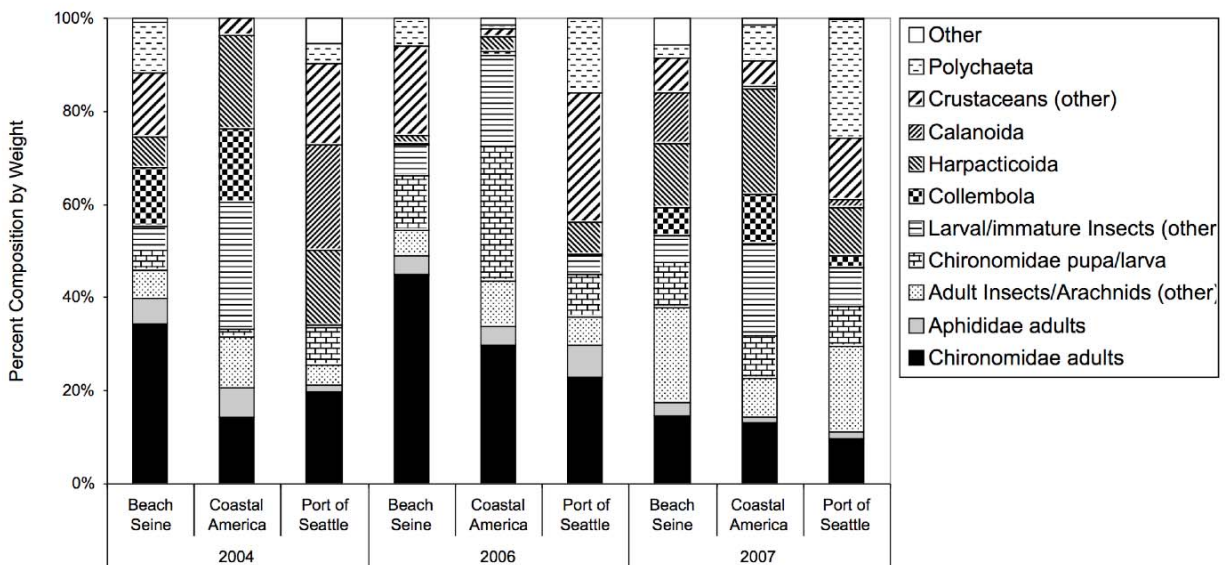
### Diet Composition

In juvenile Chinook salmon diets, adult chironomid flies were prominent at both restoration and reference beach sites (Fig. 17). Other types of adult and immature insects were also important diet components at both types of sites. *Americorophium* amphipods and other crustaceans also occurred consistently in juvenile Chinook salmon diets, especially at the beach seine site. Polychaete worms, consisting primarily of the family Nereidae, were particularly prominent at the Port of Seattle restoration site.

Diets of juvenile chum salmon were similar to those of juvenile Chinook salmon in having consistent contribution of adult chironomids across sites and times (Fig. 18). However, they differed from Chinook salmon diets in having larger proportions of larval chironomids and other immature insects and fewer adult insects, and also in having harpacticoid and calanoid copepods as prominent diet components.

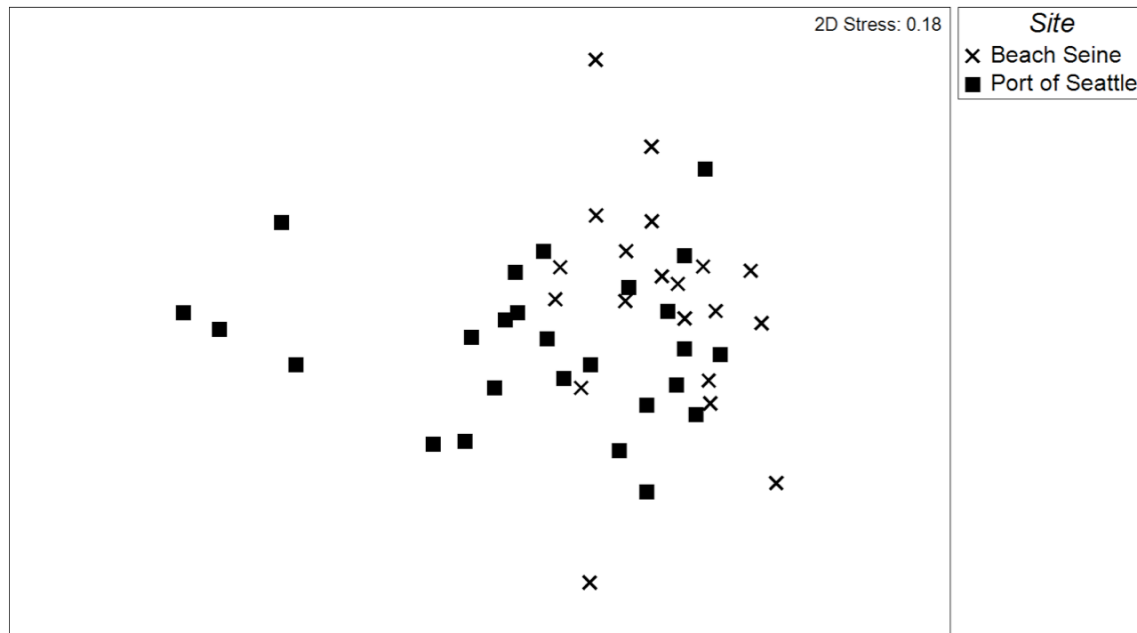


**Figure 17. Composition of juvenile Chinook salmon diets at Turning Basin restoration and reference sites, 2004-2007.**

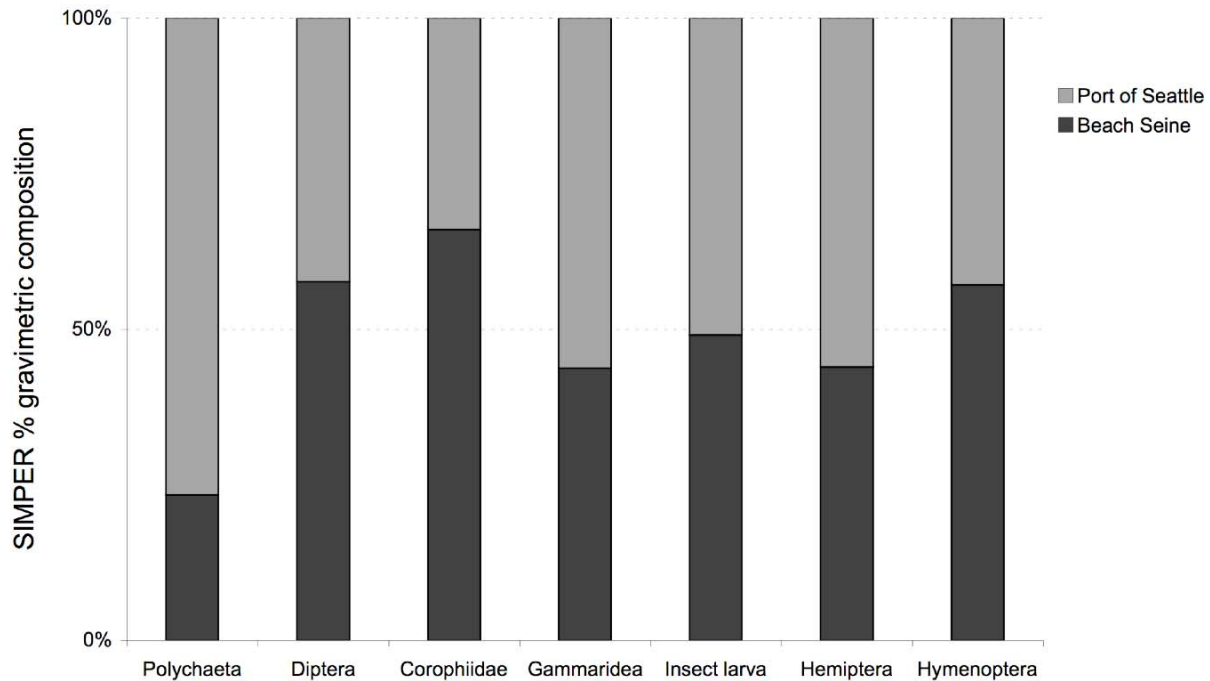


**Figure 18. Composition of juvenile chum salmon diets at Turning Basin restoration and reference sites, 2004-2007. There was no data in 2005 due to low fish numbers.**

NMDS analysis of the juvenile Chinook salmon diets based on percent composition proved to be a useful model according to statistical guidelines, with a NMDS ordination 2-d stress of 0.18 (Fig. 19). On the ordination plot, the beach seine and Port of Seattle samples clustered separately, but with some overlap (there were insufficient sample numbers from the Coastal America site to include in the analysis). These results were significant (2-way ANOSIM,  $R = 0.353$ ,  $p = 0.001$ ). A SIMPER analysis illustrated that the main differences between the two sites were high contributions by polychaete worms to the diets at the Port of Seattle site and high contributions by corophiid amphipods at the beach seine site, with other main prey taxa having similar contributions at the two sites (Fig. 20).

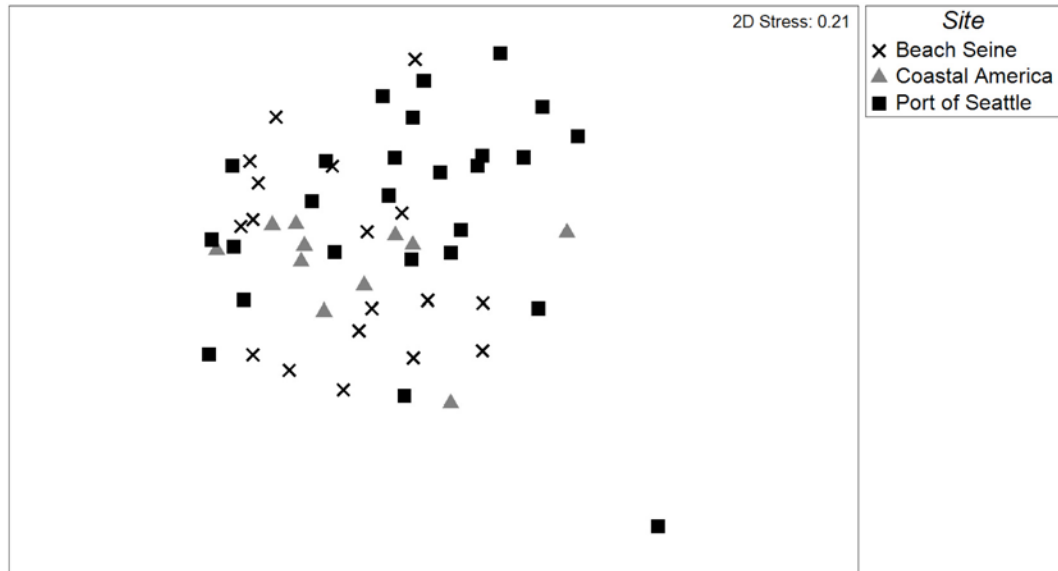


**Figure 19. NMDS ordination plot of Chinook salmon diet based on percent gravimetric composition from Turning Basin Port of Seattle and beach seine reference sites, 2004-2007.**



**Figure 20. Results from 2-way SIMPER analysis showing relative proportions of major taxa in Chinook salmon diets at Turning Basin restoration and beach seine reference sites, 2004-2007.**

NMDS analysis of the juvenile chum salmon diets based on percent composition proved to be only a marginally useful model, with a NMDS ordination 2-d stress of 0.21 (Fig. 21). Distinct site-based clusters were not evident in the ordination plot, and differences were not significant, mainly due to low R values (global  $R=0.122$ , highest R of pairwise comparisons 0.132, between beach seine and Port of Seattle sites).

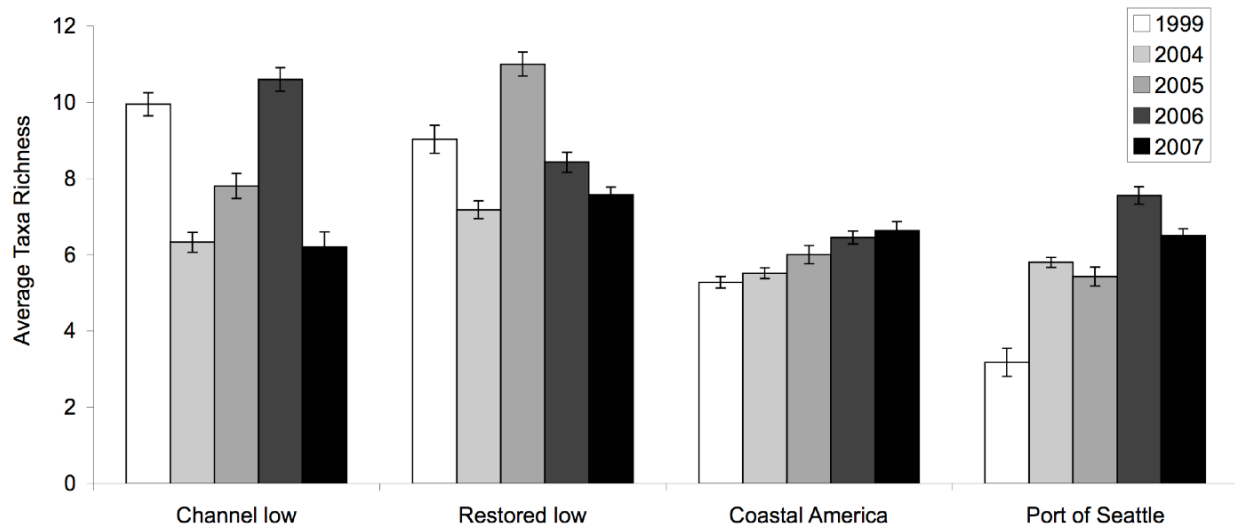


**Figure 21. NMDS ordination plot of chum salmon diet based on percent gravimetric composition from Turning Basin restoration and reference sites, 2004-2007.**

## **Benthic Macroinvertebrates**

### Taxa Richness

Average taxa richness was similar at the lower intertidal mudflats near the restored and reference sites, and among site differences were not statistically significant (Fig. 22). At the higher elevation restoration sites, a 2-way ANOVA with site and year found a significant site effect ( $p=0.0495$ ). Because there were also significant year and interaction effects ( $p<0.0001$ ), separate 1-way ANOVAS by site for each year, and by year for each site were conducted. There were significant between-site differences in 1999, when the Coastal America site had higher taxa richness than the Port of Seattle site, and in 2006, when the reverse was true. At the Coastal America site, there was a significant year effect ( $p<0.0001$ ), and a post-hoc Tukey test indicated that 2006 and 2007 had higher average taxa richness than 1999 and 2004. Similarly, year effect was also highly significant at the Port of Seattle site ( $p<0.0001$ ), and post-hoc tests indicated that 1999 had significantly lower taxa richness than all other years, and 2006 had significantly higher taxa richness than in other years.



**Figure 22.** Average taxa richness by year of benthic macrofauna at Turning Basin restored and reference sites, 1999 and 2004-2007.

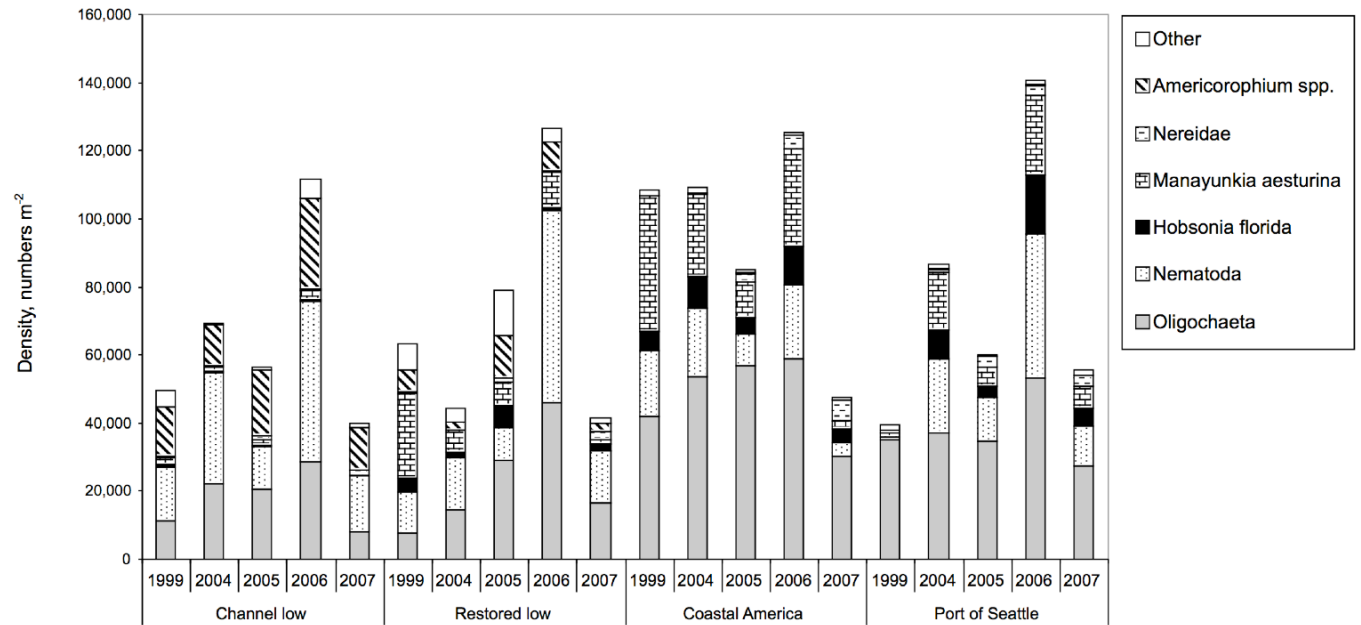
### Assemblage Compositions

Benthic macrofauna assemblages were composed mostly of oligochaetes and nematodes, and three taxa of polychaete worms: the family Nereidae and the species *Hobsonia florida* and *Manayunkia aesturina* (Fig. 23). The higher elevation Coastal America and Port of Seattle sites were different from the two lower elevation mud flat sites in having higher proportions of *H. florida* and *M. aesturina*. The lower elevation sites were characterized by the presence of *Americorophium* spp. amphipods, which were rare at the upper elevation sites.

NMDS analysis of the benthic macrofauna assemblages based on percent composition resulted in a useful model according to statistical guidelines, with a NMDS ordination 2-d stress of 0.19 (Fig. 24). On the ordination plot, the higher elevation Coastal America and Port of Seattle samples clustered separately from the lower elevation mudflat samples, and the latter two sites grouped separately from each other. Also, samples from the initial sampling of the Port of Seattle site in 1999 grouped separately from the other samples. These results were significant (2-way ANOSIM,  $R = 0.592$ ,  $p < 0.001$ ; by year,  $R=0.4$ ,  $p < 0.001$ ). ANOSIM analysis also indicated that there were significant differences in all pairwise comparisons of high elevation vs low elevation sites. Within elevation type, the higher Coastal America and Port of Seattle sites were not significantly different, but there was a difference between the two lower elevation sites. A SIMPER analysis showed that this difference was due to higher contributions by *Americorophium* spp. and juvenile corophiid amphipods at the reference channel site and more of the polychaetes *Manayunkia aesturina* and *Hobsonia florida* and the cumacean crustacean *Nippoleucon hinumensis* at the mud flat near the restoration site (Fig. 25).

In a separate NMDS analysis based on sampling year at the Port of Seattle site, SIMPER analysis showed that the difference between samples taken in 1999 and those taken in 2004-2007 was due to contributions by chironomid fly larvae and *Nippoleucon hinumensis* in the 1999 samples.

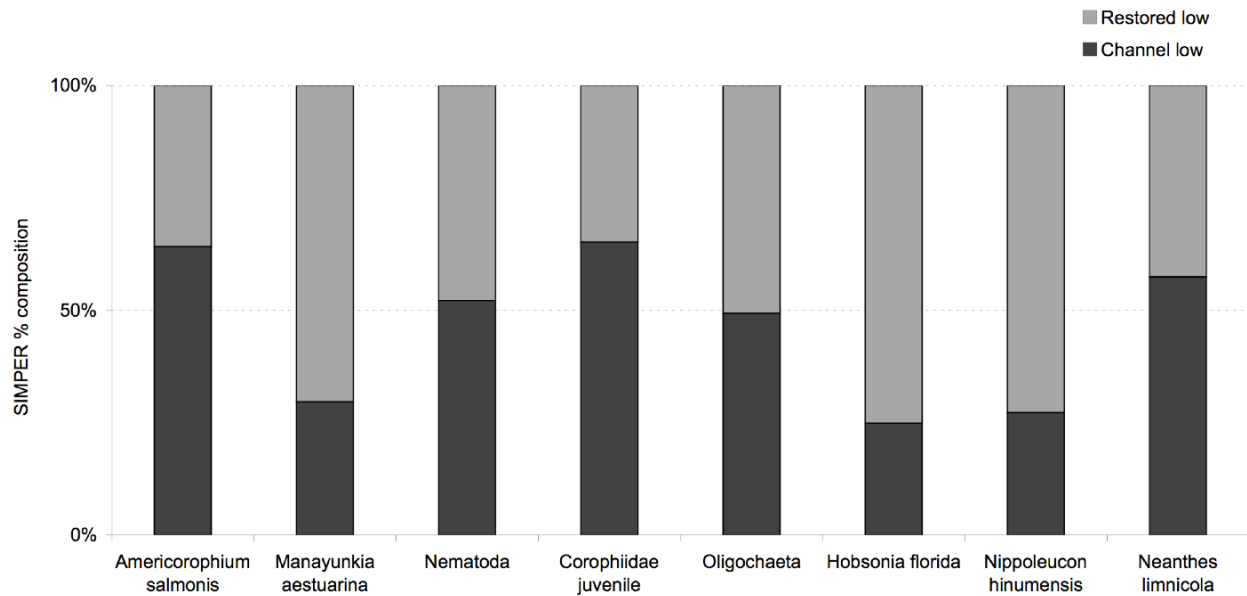




**Figure 23.** Average densities of major benthic macrofauna taxa at Turning Basin restored and reference sites, 1999-2007.



**Figure 24.** NMDS ordination plot of macrofaunal invertebrate assemblages based on percent composition from Turning Basin restoration and reference sites, 1999 and 2004-2007.

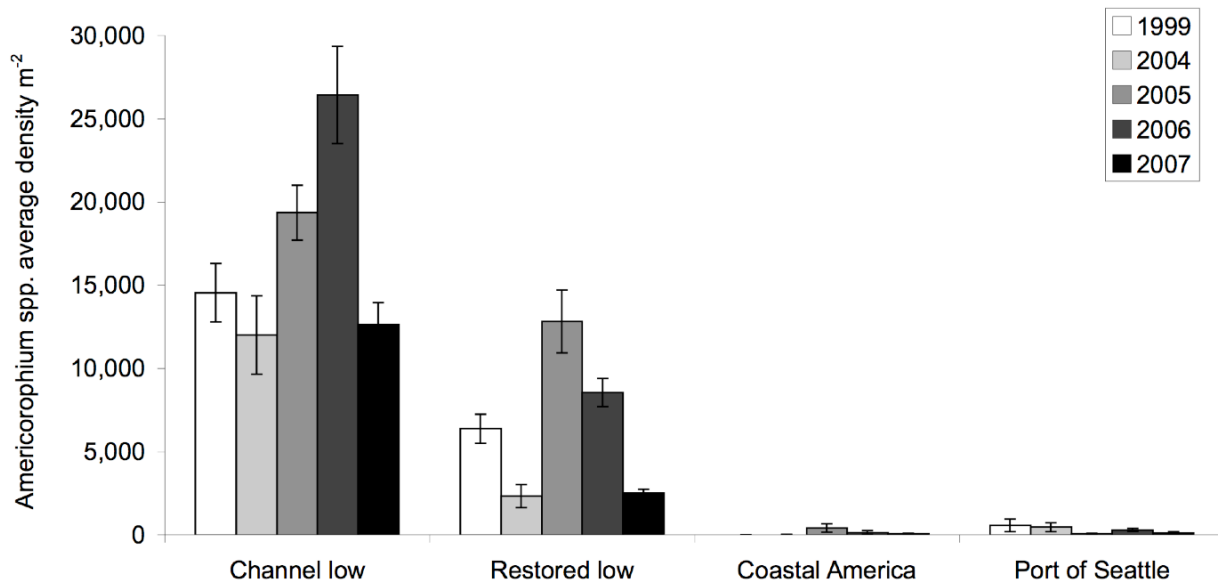


**Figure 25. Results from 2-way SIMPER analysis showing relative proportions of major benthic macrofauna taxa at Turning Basin lower elevation restoration and reference sites, 1999-2007.**

#### Densities of Important Salmon Prey Taxa

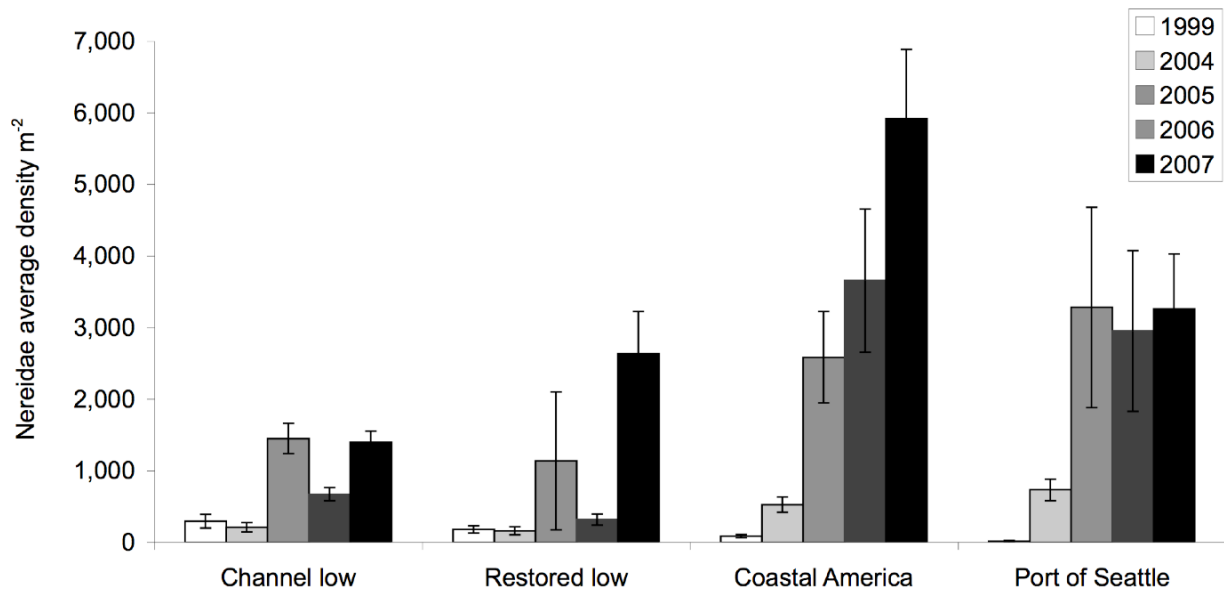
The two benthic macrofauna taxa that were most important in juvenile Chinook salmon diets were genus *Americorophium* amphipods and polychaetes in the family Nereidae.

*Americorophium* spp. occurred in very low abundance at the higher elevation restoration sites (Fig. 26), and there were no differences at this elevation among years or between sites in ANOVA analyses. At the two lower elevation mudflat sites, *Americorophium* spp. were much more abundant. At these sites, a 2-way ANOVA indicated that site and year both had significant effects on *Americorophium* densities ( $p < 0.0001$ ). Interaction effects were also significant ( $p = 0.008$ ), and post-hoc Tukey tests indicated that 2005 densities were higher than in other years. One-way ANOVAs by year indicated that in each sampling year densities of *Americorophium* were higher at the reference channel site than at the mudflat near the restoration sites.



**Figure 26. Density of *Americorophium* amphipods at Turning Basin restoration and reference sites, 1999 and 2004-2007,  $\pm$  SE.**

In each sampling year except 1999, nereid polychaetes were more abundant at the higher elevation restoration sites than at the lower elevation mudflats (Fig. 27). At the two lower mudflat sites, there were no significant differences in nereid abundances by site ( $p=0.73$ ), but there was a difference by year ( $p < 0.0001$ ), with Tukey post-hoc tests indicating that abundances were significantly higher in 2005 and 2007 as compared to other years. Similarly, at the higher elevation restoration sites, there were no significant differences in nereid abundances by site ( $p=0.31$ ), but there was a difference by year ( $p < 0.0001$ ). Post-hoc Tukey tests indicated that samples from 2005-2007 had significantly higher abundances of nereids than those taken in 1999 and 2004.



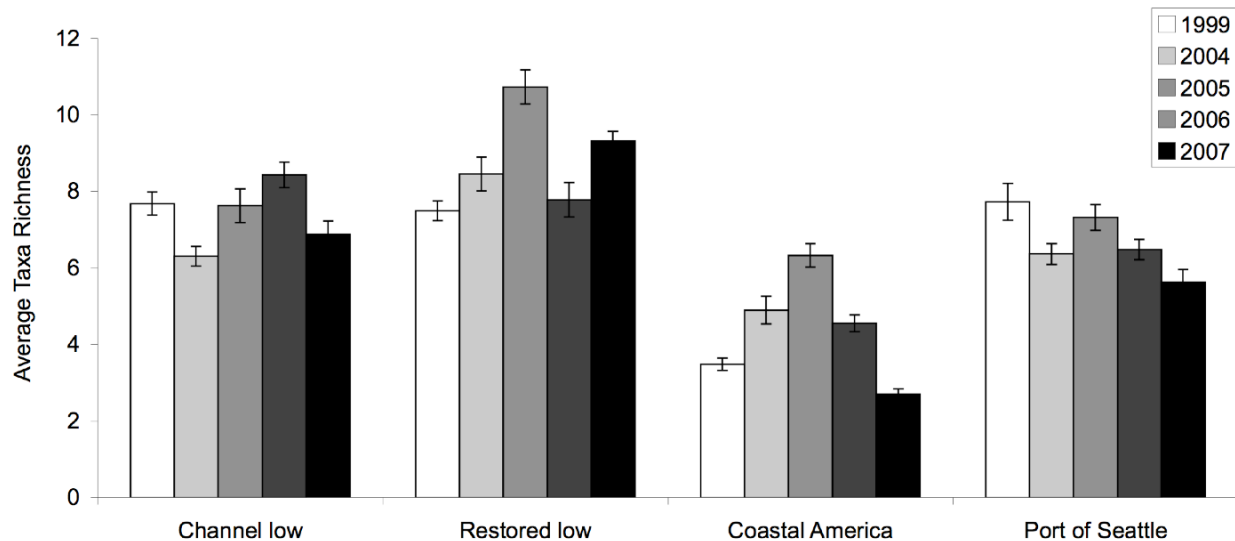
**Figure 27.** Density of nereid polychaetes at Turning Basin restoration and reference sites, 1999 and 2004-2007,  $\pm$  SE.

## Benthic Meiofauna

### Taxa Richness

In a comparison of the two lower elevation sites, there were no trends of increasing or decreasing meiofauna taxa richness through time (Fig. 28). However, a 2-way ANOVA using site and year indicated that year, site, and interactions were all significant ( $p < 0.0001$ ), i.e., some years had higher taxa richness than other years. Individual ANOVAs conducted by year indicated that for the lower elevation sites, the mudflat near the restored sites had significantly higher taxa richness than the reference channel mudflat in 2004, 2005, and 2007.

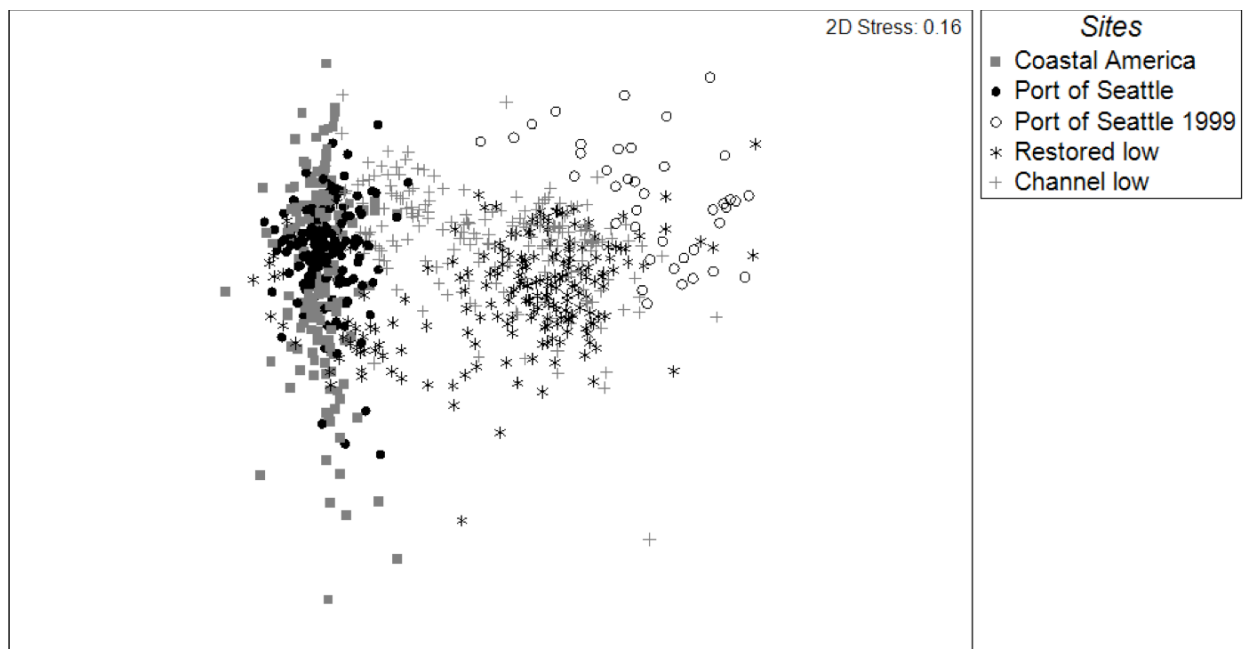
Similar to the lower elevation sites, a 2-way ANOVA conducted on meiofauna samples from the higher elevation restoration sites found that year, site, and interactions were all significant ( $p < 0.0001$ ). At the Port of Seattle site, post-hoc Tukey tests indicated that samples from 1999 had higher taxa richness values than those from 2004 and 2007. The Port of Seattle site had significantly higher taxa richness than the Coastal America site in each sampling year ( $p < 0.05$ ).



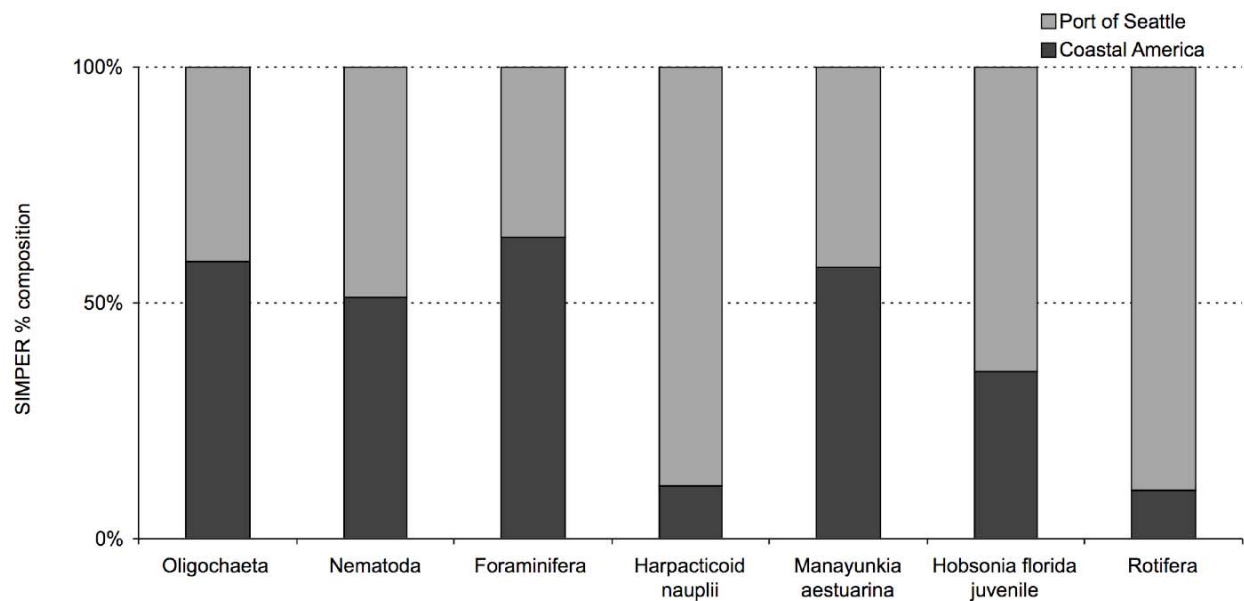
**Figure 28.** Average taxa richness by year of benthic meiofauna at Turning Basin restored and reference sites, 1999 and 2004-2007.

### Assemblage Compositions

NMDS analysis of the benthic meiofauna assemblages based on percent composition resulted in a useful model according to statistical guidelines, with a NMDS ordination 2-d stress of 0.16 (Fig. 29). Similar to the results from the benthic macrofauna samples, on the ordination plot the higher elevation Coastal America and Port of Seattle samples clustered separately from the lower elevation mudflat samples, and samples from the 1999 Port of Seattle site grouped separately. These results were significant (2-way ANOSIM, site  $R = 0.563$ ,  $p < 0.001$ ; by year,  $R=0.372$ ,  $p < 0.001$ ). The Port of Seattle and Coastal America sites were also different from each other ( $R=0.37$ ,  $p < 0.001$ ). A SIMPER analysis showed that the difference between the Port of Seattle and Coastal America restoration sites was due to higher contributions by harpacticoid copepod nauplii (larval) stages and rotifers at the Port of Seattle site (Fig. 30). Additional NMDS analyses indicated that these taxa differences stemmed almost entirely from 1999 samples.



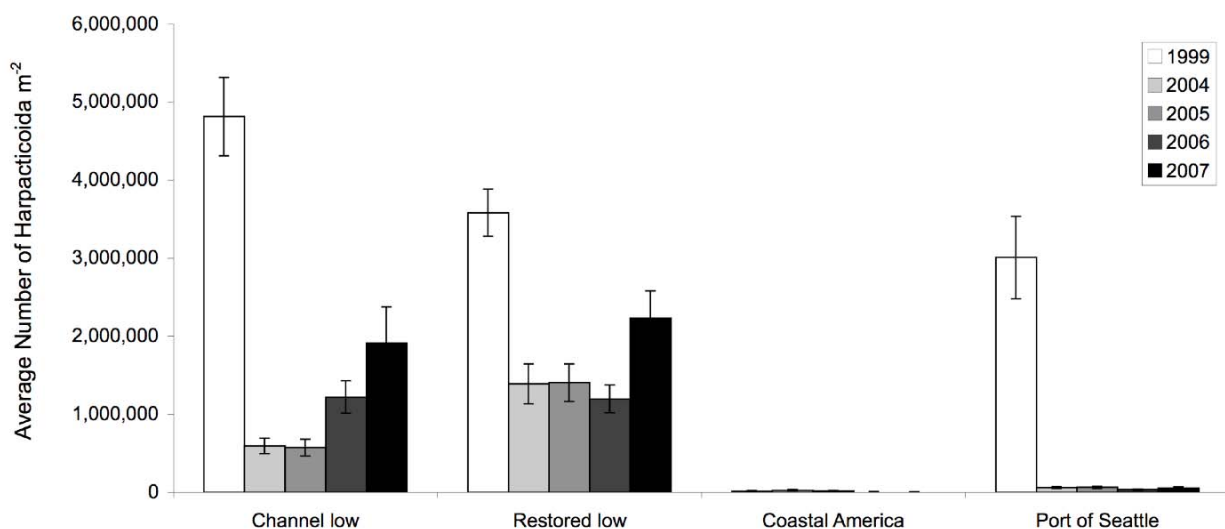
**Figure 29. NMDS ordination plot of meiofaunal invertebrate assemblages based on percent composition from Turning Basin restoration and reference sites, 1999 and 2004-2007.**



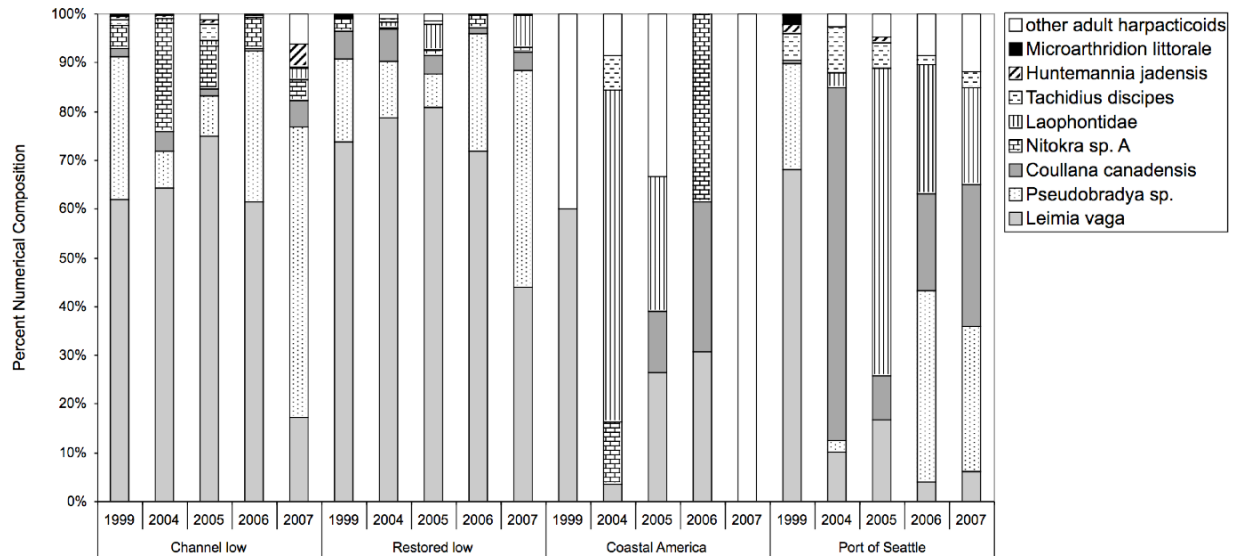
**Figure 30. Results from 2-way SIMPER analysis showing relative proportions of major benthic meiofauna taxa at Turning Basin higher elevation restoration and reference sites, 1999-2007.**

### Densities of Important Salmon Prey Taxa

Harpacticoid copepods, which were the main meiofaunal group that was important in juvenile chum salmon diets, were much more abundant at the lower elevation sites with the exception of 1999, when there was a large recruitment of harpacticoids at the higher elevation Port of Seattle site (Fig. 31). Results from a 2-way ANOVA on the two lower elevation sites using site and year found significant year ( $p < 0.0001$ ) and interaction ( $p = 0.0036$ ) effects. Post-hoc Tukey tests indicated that for these two sites 1999 had higher harpacticoid densities than all other sampling years, and also that 2007 samples at the channel site had higher densities than those from 2004 and 2005. Harpacticoid copepod assemblages were also different in composition between the higher and lower elevation sites, which may have contributed to their clustering separately in the ordination plot (Fig. 32). The lower elevation sites were always dominated by *Leimia vaga* and *Pseudobradia* sp., while the higher elevation restoration sites had much less dominance by one or two taxa, and greater contributions by *Coullana canadensis*, Laophontidae, and *Nitokra* spp.



**Figure 31. Density of harpacticoid copepods (naupliar stages omitted) at Turning Basin restoration and reference sites, 1999 and 2004-2007,  $\pm$  SE.**



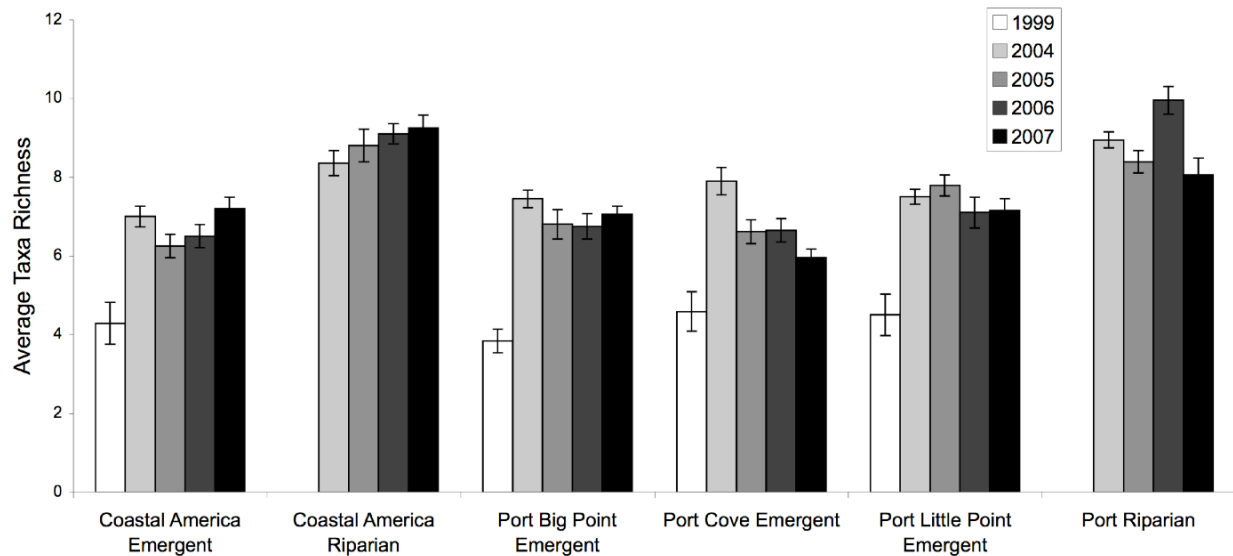
**Figure 32. Percent composition by numbers of harpacticoid copepod taxa at Turning Basin restoration and reference sites, 1999 and 2004-2007.**

## Fallout Insects

### Taxa Richness

Taxa richness was analyzed at the order level to be consistent with the level of taxonomic identification used across all sampling years. With the exception of low taxa richness in 1999 samples, there were no consistent trends across sampling periods (Fig. 33). Emergent vegetation, which was the only stratum that was sampled consistently in all years, was analyzed with a 2-way ANOVA using site and year. The results indicate that year effect was significant ( $p < 0.0001$ ), and site and interaction effects were not significant. A Tukey post-hoc test indicated that 1999 samples had lower taxa richness than all other sampling years. Riparian and emergent strata could be compared within each site for the years 2004-2007. At the Coastal America site, in a 2-way ANOVA using stratum and year comparing fallout samples from emergent and riparian vegetation strata, stratum effect was significant, with riparian samples having more taxa than emergent samples ( $p < 0.0001$ ), and year and interaction effects were not significant. Similarly, at the Port of Seattle site, stratum effect was significant, but year and interaction effects were also significant ( $p = 0.003, 0.0005$  respectively), indicating some interannual variation and variation in the three emergent and one riparian strata. Post-hoc Tukey tests indicated that in each sampling year, riparian vegetation strata had higher taxa richness than the emergent vegetation strata.



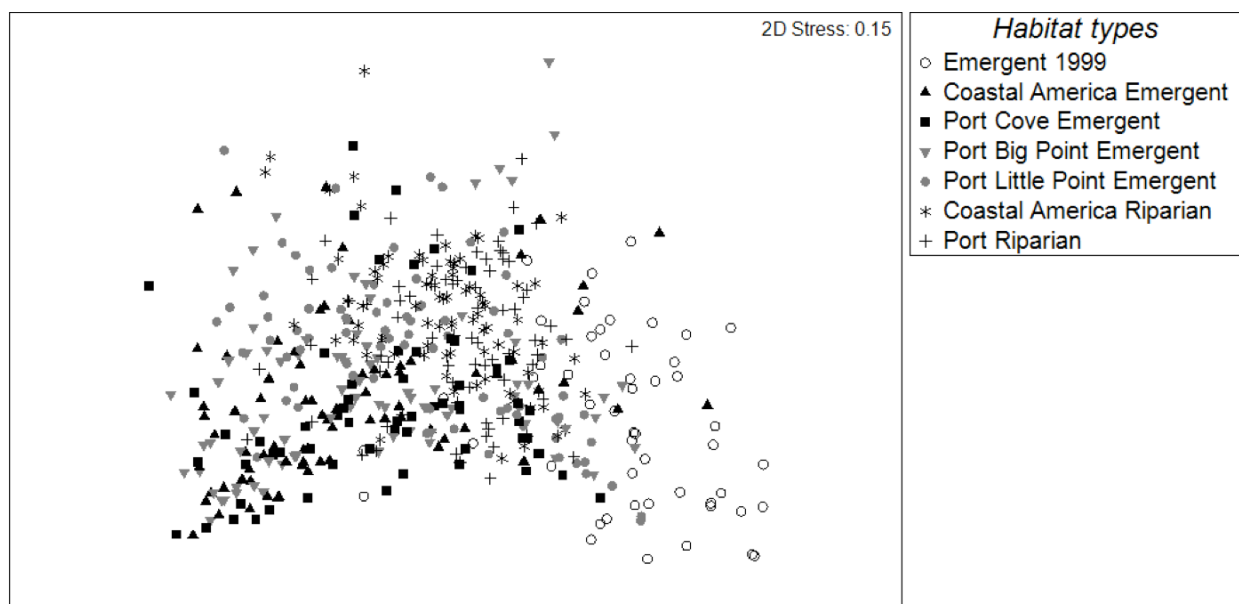


**Figure 33. Average taxa richness by year of fallout trap invertebrates at Turning Basin restored sites, 1999 and 2004-2007.**

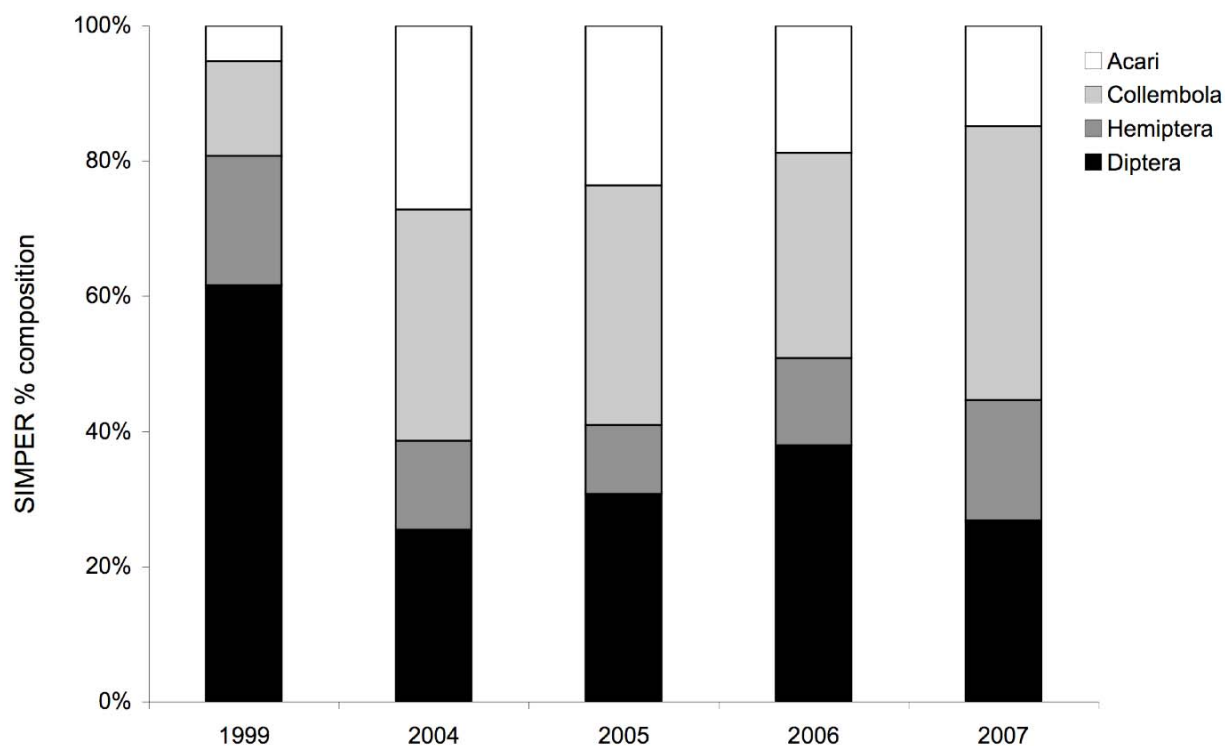
### Assemblage Compositions

NMDS analysis of the fallout trap invertebrate assemblages based on percent composition resulted in a useful model according to statistical guidelines, with a NMDS ordination 2-d stress of 0.15 (Fig. 34). On the ordination plot, emergent vegetation strata sampled in 1999 clustered separately from other samples. Overall emergent results were not significant because of low R-values (2-way ANOSIM with site and year, year  $R = 0.282$ , site  $R = 0.158$ , both  $p < 0.001$ ). However, pairwise comparisons by year indicated that samples from 1999 were different than those from other sampling years ( $R$  range from 0.41 – 0.76, other year-to-year comparisons,  $R < 0.25$ ). A SIMPER analysis showed that the difference between 1999 fallout trap samples and those from other sampling years was due to higher contributions by dipteran flies in 1999, and higher contributions by Acari (mites) and collembolans in other sampling years (Fig. 35).

Pairwise comparisons could be made of riparian and emergent strata at each site for the years 2004-2007. At the Coastal America site, the two strata were different ( $R = 0.446$ ,  $p < 0.001$ ). Riparian and emergent strata at Port of Seattle were less significant, with  $R$  values of 0.3 – 0.37 in comparisons of the three emergent and one riparian strata. SIMPER analysis indicated that the riparian strata had more hymenoptera and arachnids than the emergent strata.



**Figure 34.** NMDS ordination plot of fallout trap invertebrate assemblages based on percent composition from Turning Basin restoration sites, 1999 and 2004-2007.

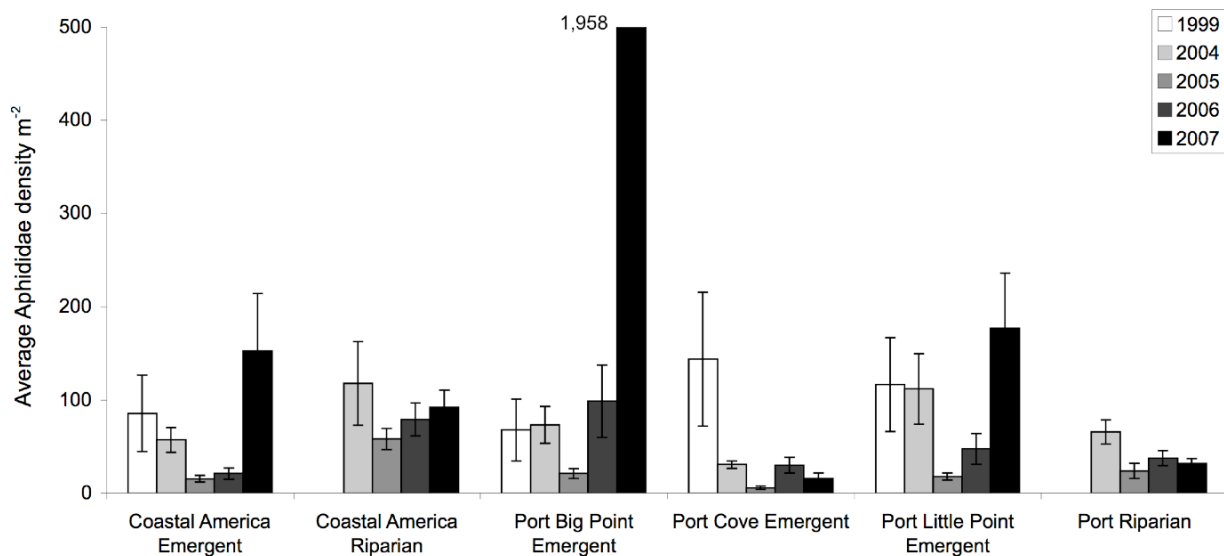


**Figure 35.** Results from SIMPER analysis showing relative proportions of major emergent vegetation fallout trap invertebrate taxa at Turning Basin higher elevation restoration sites, 1999-2007.

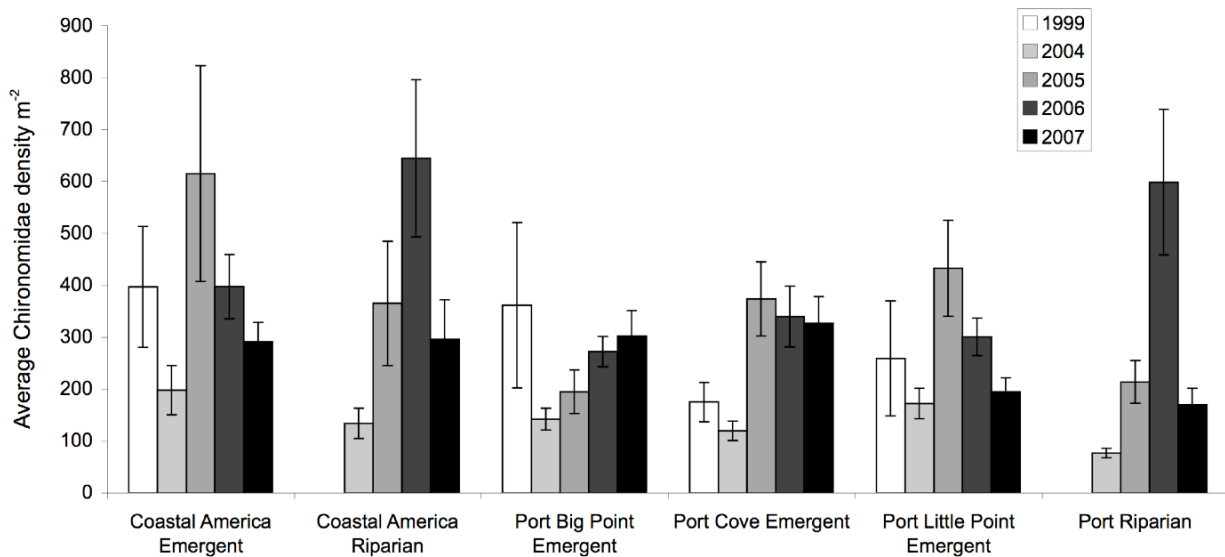
### Densities of Important Salmon Prey Taxa

Aphids were identified to family level consistently across sampling years, and also occurred consistently in both juvenile chum and Chinook salmon diets (Figs. 17, 18). There were no evident trends of increasing or decreasing abundances of aphids across sampling years, but there were particularly high abundances in 2007 at three of the six sampling sites, especially at the “Big Point” emergent vegetation stratum at the Port of Seattle restoration site (Fig. 36). A 2-way ANOVA for the emergent vegetation strata using site and year found that site, year and interaction terms were all significant ( $p < 0.001$ ), indicating considerable among site- and year variation. A post-hoc Tukey test showed that 2007 had higher aphid densities than all other sampling years, due to the extremely high numbers at the “Big Point” site in that year. At the Coastal America site, there was no significant difference in aphid densities between emergent and riparian strata. At the Port of Seattle site, sampling strata, year, and interaction effects were all significant ( $p < 0.001$ ), but in pairwise comparisons of emergent vs riparian strata, post-hoc Tukey tests indicated that significant differences were driven by the high aphid numbers only at the “Big Point” emergent stratum, which had significantly higher abundances than all other strata.

Adult chironomid flies were also important and consistent prey items in both chum and Chinook salmon diets. At emergent and riparian vegetated strata, there were no evident trends of increasing or decreasing abundances of chironomids across sampling years (Fig. 37). A 2-way ANOVA conducted on fallout trap samples from the emergent vegetation strata found that both site and year were significant ( $p = 0.04, 0.0002$  respectively). Post-hoc Tukey tests did not find any sites or sampling years that were consistently different from all of the other sites and years: the Coastal America emergent vegetation site had higher chironomid densities than the Port of Seattle “Big Point” emergent vegetation site, and samples from 2004 had lower densities than those from 2005 and 2006.



**Figure 36. Density of Aphididae at Turning Basin restoration vegetation strata, 1999 and 2004-2007,  $\pm$  SE.**



**Figure 37. Density of Chironomidae at Turning Basin restoration vegetation strata, 1999 and 2004-2007,  $\pm$  SE.**

## Discussion

### Fish Assemblages

Juvenile salmonids were found at all three fish-sampling sites, although in different proportions and with different size distributions. The Port of Seattle restoration site and the reference beach seine site had significantly higher densities of Chinook salmon than the Coastal America restoration site. There were no significant differences among the three sites in chum salmon densities, but both chum and Chinook salmon sizes were nearly always smaller at the Coastal America site. Taxa richness and multivariate analyses further accentuated the differences among the sites: The Port of Seattle restoration site had significantly higher taxa richness, while the Coastal America site had significantly lower taxa richness. NMDS analysis found that the three sites had different fish assemblages and that the main pattern driving the differences were low or no contributions of Chinook salmon, coho salmon, shiner perch, starry flounder, and steelhead trout at the Coastal America site. These data suggest that restoration site elevation and/or size plays an important role in determining which salmon and other species and life-history stages occupy and actively use a site. It appears that deeper, larger sites support use by more species of salmonids and other fish than do shallower, smaller sites. Restored site access in a tidal system is a function of not only water depth but also of inundation time: higher elevation sites such as the Coastal America site are underwater for less time than lower elevation sites. Thus, in addition to having reduced taxa diversity, higher elevation sites are available for less time to those species that use the site. However, these findings would not preclude the possibility that smaller, shallower sites can benefit the estuarine food web through export of organic material and prey (e.g., insects) to the system. In fact, export of organic material has been the stated goal of several restoration sites in the Duwamish Estuary, but this attribute has rarely been measured (Armbrust et al. 2009).

## **Juvenile Salmon Diets**

Juvenile Chinook salmon diets in this study were similar to those found in diets from other natural and restored wetland sites in the Pacific Northwest, consisting mostly of chironomids, a variety of other insects, and corophiid amphipods (Levings et al. 1995, Shreffler et al. 1992, Miller and Simenstad 1997, Tanner et al. 2002). Nereid worms were also an important diet item, especially at the Port of Seattle restoration site, and they have also been found in earlier studies in the Duwamish estuary and in diets of juvenile Chinook salmon rearing in the Puget Sound nearshore (Cordell et al. 2001, Brennan et al. 2004). The main finding of the NMDS analysis of Chinook salmon diets was that differences between the beach seine reference site and the Port of Seattle restoration site were mainly due to higher proportions of nereid polychaetes at the Port of Seattle site and more corophiid amphipods at the beach seine site. This corresponds to the results from the benthic invertebrate sampling that showed few amphipods and more nereids at the Port of Seattle site compared to the channel mudflat site.

Juvenile chum salmon prey overlapped with Chinook salmon prey, primarily in the presence of adult chironomids and nereid polychaetes, which were sometimes important prey for both species. Chum salmon diets differed from Chinook salmon diets in having more small crustaceans such as harpacticoid and calanoid copepods and more larval and pupal chironomids. In the latter respect, chum salmon diets were similar to those using a restored wetland area in the Puyallup River estuary, where Shreffler et al. (1992) found that chum salmon tended to eat more chironomid larvae than Chinook salmon. Chum salmon are also known to feed extensively on chironomids in several regional estuarine natural and restored marshes (e.g., the Skagit estuary—Congleton 1978, and the Snohomish estuary—Tanner et al. 2002). Unlike Chinook salmon, NMDS analysis found no large differences between the diets of juvenile chum salmon among the sampling sites. The exact reasons for this are not known, but one possibility is that, in contrast to between-site differences seen for benthic macrofaunal Chinook salmon prey, the types of prey that chum salmon feed on are more evenly distributed among the sites sampled (see under Benthic Meiofauna, below).

Instantaneous rations found in juvenile chum and Chinook salmon in this study show that the fish feeding within the restoration sites are obtaining amounts of prey equal to or higher than those outside restoration sites. This was especially true for Chinook salmon, which had significantly higher overall instantaneous rations at the Port of Seattle site as compared to the reference beach seine site. Determining whether or not the higher instantaneous rations obtained at the restored sites translates into higher growth rates for the fish would depend on the energy content of the prey obtained at the site. For example, annelid worms, which were typical of prey at the Port of Seattle restoration site have only about 50% of the energy content of corophiid amphipods, which were more typical of diets and benthic samples from the reference beach seine site (Gray 2005, Cordell et al. 2006).

## **Benthic Macroinvertebrates**

At the Port of Seattle site, taxa richness increased significantly between the first sampling event in 1999 and the next one in 2004, to levels that comparable to those at the older Coastal America site. These results are in line with those from studies of benthic invertebrates in *Spartina* marshes in southern California and North Carolina that found that after 4-5 years benthic taxa richness in restored sites was similar to reference sites, and that early colonizers had been replaced by more typical marsh fauna (Levin et al. 1996, Talley and Levin 1999, Craft et al. 2003). However, reaching densities and invertebrate community structure similar to reference

levels can take longer in these habitats. For example in several cases in which *Spartina* marshes were restored at a much larger scale than the sites in this study, it took 10-15 years to reach reference levels for these attributes (Talley and Levin 1999, Craft et al. 2003). Unfortunately, there were no within-system natural reference sites for us to compare to the restoration sites, and we cannot conjecture as to whether or not abundances and assemblage structures have reached those that would be natural in the Duwamish estuary. Total invertebrate densities in this study fell within the range of densities of benthic invertebrates previously found at restored and natural reference sites in the Snohomish River estuary, but differed from those sites in having many fewer chironomid and ceratopogonid larvae in the samples (Cordell et al. 1998). The reason for lack of insect larvae in benthic samples from the Turning Basin restored sites may be lack of organic matter: the Snohomish estuary restoration sites had been freshwater impounded wetlands before restoration, and had thus built up considerable organic matter. Because organic matter is one attribute that may take decades to reach natural levels in restored estuarine sites (Craft et al. 2003), the Turning Basin sites may not have accumulated enough organic matter at the present stage to support larval insects.

The higher elevation Port of Seattle and Coastal America restoration sites had different assemblages of organisms than the lower elevation restoration and reference mudflats. This was evidenced by the NMDS analysis which found that the two types of sites clustered separately. The higher sites had more nereid and other polychaete worms, and the lower sites had more *Americorophium* spp. amphipods. These differences were reflected in Chinook salmon diets from the two sites: there were more polychaetes (primarily nereids) in diets from the Port of Seattle site, and more *Americorophium* in diets from the beach seine site.

There were few differences in benthic invertebrates between the Coastal America and Port of Seattle restored sites. At both of the sites, salmon prey polychaetes in the family Nereidae increased significantly from the earlier sampling periods. Along with the increases in taxa richness, this suggests that the Port of Seattle site has reached equivalency with the older Coastal America site, and that both sites have continued to develop. The benthic invertebrate assemblage at the Turning Basin site changed substantially between 1999 and 2004 from being dominated by oligochaetes to an assemblage distributed more evenly into a number of categories. In this respect, it is similar to other created marsh habitats, where dominance by a few oligochaete and insect taxa characterizes the early successional state (e.g., *Spartina* marshes in the Tijuana estuary—Moseman et al. 2004, *Salicornia* marshes in southern California—Talley and Levin 1999). At the lower elevation sites, NMDS analysis found that the channel site and the restoration site were different: the channel site had higher densities of *Americorophium* spp, and the restoration site had higher densities of *Manayunkia aesturina* and *Hobsonia florida* polychaetes as well as cumaceans. The differences may not be due to the restored- non-restored state of each site, because the elevation of the actual restoration construction was higher than the mudflat site, which was relatively unchanged from the pre-restoration state. It is more likely that the differences were due to location with regard to the river channel: the reference site was situated in a higher flow area on the main channel of the estuary, while the restoration lower elevation site was off-channel. Thus, hydrological conditions associated with each site probably created physical conditions (that we did not measure) that determined the invertebrate assemblages.

Some taxa of juvenile salmon prey, such as corophiid amphipods, have not colonized the higher elevation restored habitats in numbers as high as those found at the lower elevation mudflat sites. As the sites continue to mature, they may become more diverse and similar to the

reference sites. In one study of *Salicornia* marshes, differences in invertebrate composition at restored and reference sites converged only after 10 years (Levin and Talley 2002). Based on previous work in the Duwamish Waterway (Cordell et al. 2001), colonization of the sites by typical estuarine biota might occur within five to seven years. However, differences between the restored and reference sites may be the result of a number of factors, some of which are not related or only peripherally related to the effects of the restoration. The most important of these factors is elevation, and we do not expect complete convergence between the higher elevation restoration sites and the lower elevation sites. Our ordination analysis corroborates this in finding that the community structure was quite different at higher elevation sites as compared to lower elevation sites. However, we note that restored sites elsewhere in the Duwamish Waterway that have elevations similar to the Turning Basin Port of Seattle restored mudflat had relatively high densities of *Americorophium* amphipods within several years of construction (e.g., Terminal 105 and GSA sites—Cordell et al. 1999). Determining the sources of differences between the restored and reference sites would require continued sampling across time to look for convergence between the two site types, and/or more intensive sampling of biotic and abiotic factors such as organic material, grain size, submergence time, and flow regime. It may also be helpful in future sampling to add reference sites at higher elevations, if suitable undisturbed habitat can be found in close proximity to the restored sites.

### **Benthic Meiofauna**

The finding that meiofaunal taxa richness did not increase with time at the two restoration sites suggests that initial colonization by this group was rapid. This is further corroborated by the harpacticoid copepod density data, that shows a large recruitment of harpacticoids at the Port of Seattle site just after construction in 1999. This is not surprising, given recent experimental results using artificial substrata that show that harpacticoids colonize new substrata in as little as a few hours, and quickly reach high densities (Atilla and Fleeger 2000, Chertoprud et al. 2005). Chertoprud et al. (2005) found that the earliest colonizing harpacticoids were epibenthic forms that could swim and that were transported via water currents, and the early colonizers in 1999 at the Port of Seattle site were also epibenthic harpacticoids (data not included in this report, but available on request). High harpacticoid abundances were also seen in 1999 at the two lower elevation sites, but not at the higher elevation Coastal America site. The reasons for the low 1999 numbers at the latter site are unknown, but may be related to its elevation or age. In subsequent sampling years, harpacticoid numbers at the Port of Seattle site were low, and the upper elevation sites appear to have reached post-colonization harpacticoid numbers that are much lower than those at the lower elevation sites.

### **Fallout Insects**

At the emergent vegetation restoration sites that we sampled, insect taxa richness increased significantly between 1999 and the next sampling in 2004, and remained high. These results are not surprising given that insects can rapidly colonize restored vegetated habitats. For example, after the eradication of invasive *Phragmites* grass in a *Spartina* marsh in New Jersey, insect and spiders returned rapidly to the newly recruiting *Spartina*, and in less than five years were indistinguishable from reference marshes in taxa richness and assemblage structure (measured using NMDS) (Gratton and Denno 2005). We found significantly higher taxa richness at the restored riparian vs the emergent vegetation strata. This may be related to the plant communities within each stratum: in the emergent areas, plants are dominated by only two species, the sedge

*Carex lyngbei* and *Scirpus* sp., while the riparian areas were planted with a variety of trees and shrubs.

We did not detect many significant differences in fallout trap insects among sites and sampling years, or note any abundance trends across time for fallout trap insect assemblages. Likewise, for the two taxa of potential salmon prey that we examined, no site differences or trends were observed. Despite these findings, maturation of the riparian and emergent plant communities at the Port of Seattle and Coastal America restoration sites may have increased the overall insect production at the sites. Trees and shrubs, and emergent vegetation have grown and spread such that vegetation now occurs in continuous stands in many of the planted areas. Insect sampling in the new riparian growth at both of the sites was added in 2004, because it has obviously become a potentially important stratum at the shore-water interface. The high among-year variability and large sample variances (see standard error bars in Figs. 36, 37) suggest that fallout traps may not be the best way to measure insect production from smaller scale restored wetland vegetation sites. Close proximity of the sites to each other may have resulted in the fallout insects being mixed together by wind or air currents before reaching the ground, thus compromising site fidelity. Another method that has proved successful elsewhere is suction sampling such as that conducted by Gratton and Denno 2005 using a D-Vac vacuum insect net (Rincon-Vitova Insectaries, Ventura, CA, U.S.A.).

## Conclusions

In an industrialized setting such as the Duwamish estuary, there is limited land available for restoration, and results such as those from this study can help to determine if relatively small restoration sites located in a degraded landscape can benefit important resources (in this case, juvenile salmon).

Results from this study indicate that the Coastal America and Port of Seattle Turning Basin restoration sites are providing significant habitat attributes for juvenile salmon. The Port of Seattle site had similar or higher salmon densities and fish taxa richness compared to the non-restored channel site. The Coastal America site, while having lower taxa richness and low densities of Chinook salmon, had densities of chum salmon comparable to the other sites. The salmon accessing the restoration sites were obtaining prey typical of that found in other restored and natural habitats in the region. In addition, high measures of instantaneous ration in fish using the restored sites suggests that the salmon there acquire prey in amounts exceeding those in the main river channel.

At the Port of Seattle site, benthic invertebrate taxa richness increased significantly between the first sampling event in 1999 and the next one in 2004, to levels comparable to those at the older Coastal America site. At both sites, total invertebrate densities in this study fell within the range of densities of benthic invertebrates previously found at other restored and natural reference sites in the region, but differed from those sites in having fewer chironomids and other insect larvae. The reason for this may be because organic matter has not yet built up at the sites.

Initial colonization of meiofaunal harpacticoid copepods at the Port of Seattle site was rapid: meiofaunal taxa richness reached stable levels within the first sampling year, and density data showed a large recruitment of harpacticoids at the Port of Seattle site just after construction in 1999.

Insects also apparently colonized both of the restoration sites quickly, with significant increases in taxa richness between the first sampling in 1999 and the next sampling in 2004.



Planted riparian areas at the restoration sites had especially high taxa richness, which may have resulted from the diverse riparian plant assemblage that has developed.

Overall results indicate that the restoration sites at the Turning Basin have developed into productive habitats, that contribute to the opportunity for juvenile salmon feeding in the transition zone of the Duwamish estuary. Our data suggest that several physical factors are important in how the restoration sites have developed, and these should be addressed in the design of future restoration sites. They include: (1) tidal elevation of created features, (2) size of created areas, and (3) access of restored sites to the main waterway. These physical factors can influence the ecological development of restored sites, separate from the amount of time that it takes natural processes to develop. With the absence of natural habitat in this industrialized estuary, the best strategy for restoration may be to provide a diversity of sites that will provide natural features that are different from the status quo of heavily altered and channelized shoreline. Continued monitoring of the development and long-term stability of restored sites will help inform the design of improved sites for future restoration.

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